Recent Advances in
Nutrition

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with
Particular Reference to Protein Metabolism
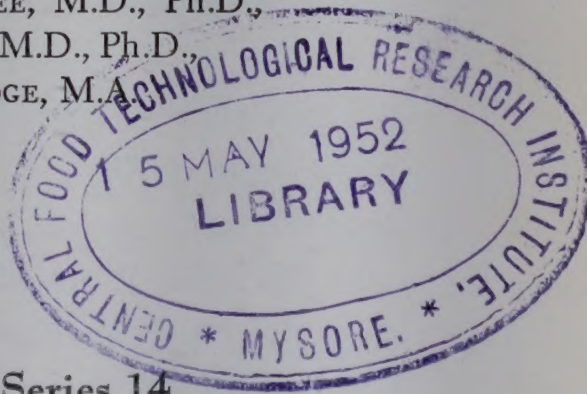
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Preface

SHORTLY AFTER the onset of the Second World War my collaborators and I became interested in the possible effects of severe and prolonged protein deficiency and its associated loss of bodily protein stores, upon resistance to infection. Early in the course of these studies we found the protein-depleted rat a useful animal for the determination of antibody formation and for the demonstration of varying grades of resistance to bacterial infection. Later we demonstrated that this type of animal is also an excellent biologic testing-medium for the evaluation of the nutritive potentialities of protein. Soon thereafter we began a collaborative study with the Quartermaster Department of the U. S. Army, directed at nutritional problems of military interest. Since the end of the war we have operated, also, as a Task Force for the Office of Naval Research of the U. S. Navy.

These lectures were presented to the faculty and students of the University of Kansas and are printed essentially as they were given. However, new material has been added where necessary in order to cover some later developments. The first lecture was addressed to undergraduate students; the second and third to the faculty and students of the School of Medicine. In them I am spokesman for a group of associates whose generous and enthusiastic coöperation I gratefully acknowledge. The opinions expressed, however, are mine, and do not necessarily reflect those of my collaborators.

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Recent Progress in Nutrition

I

Recent Progress in Nutrition

NUTRITIONAL PROGRESS in recent years has come so rapidly that even experts in the field have had but little time to evaluate its full significance. Although many of the advances may be attributed to the war and its associated nutritional needs, several important discoveries antedated the onset of the Second World War. All, however, have served to emphasize the dominant role of food and nutrition in world affairs, especially in relation to agriculture, economics, science, medicine, politics, and ethics. Indeed food scarcity or the fear of it, together with undernutrition, constitutes a major problem of our time. Because of the enormous productive potential of this country it is easy to overlook the fact that in some other areas the thought of food still dominates the daily life; and although the post-war rehabilitation plans prevented the development of actual famines, economic uncertainties doubtless will continue for many years to interfere with the hopes for better nutrition in many parts of the world.

The response of the United States to the immediate post-war needs was both generous and comprehensive; in fact, the preponderant cost of UNRRA was borne by us. Additional aid was given, also, by religious and philanthropic agencies and by many individuals. Moreover, information revealed by our wartime nutritional researches was transmitted widely, to friend and foe alike. But de-

spite our leadership, too often we have set a bad nutritional example. For example, although we all recognize the fact that only a good diet can enable our bodies to function effectively, whether in the performance of work or for the maintenance and repair of metabolic mechanisms, nonetheless we handle many aspects of our food supplies in strange and careless ways. Otherwise how can we be so complacent about our wasteful habits of overmilling, overprocessing, overcooking, and overpricing? Only in time of war do we seem to realize fully the basic significance of our food supply. Then we set up intricate rationing systems and invent attractive slogans, such as, for example, "Food Will Win the War." But after the war is over we revert quickly to our former careless ways. For instance, we allude to bread as our "staff of life" and at the same time we weaken this "staff" by the overmilling of our wheat and corn. As a consequence we feed the middlings to the pigs and poultry, and manufacture niacin to treat pellagra. We overmill our rice, discarding the vitamin-containing portions, and buy thiamine to treat beriberi. We overcook our vegetables and discard the "pot liquor," along with important salts and vitamins. We allow the price of butter to rise and then, by taxation, we discourage its replacement with oleomargarine. We overprocess some of our breakfast foods and rely upon the use of milk to counteract the damage done. We substitute alcohol for nutritious food and, often when it is too late, seek medical aid for the treatment of neuritis and cirrhosis of the liver. We eat huge quantities of candy, perhaps thereby accelerating the onset of diabetes and tooth decay. After doing these things we attempt to atone

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in some degree for our nutritional misdemeanors by the consumption of large quantities of vitamin pills. Finally, we "enrich" our bread and initiate educational campaigns to encourage consumption of "protective" foods when, in reality we should be taking appropriate measures to protect ourselves against our own shortcomings.

Regrettable as it is that we do these things, the fact cannot be denied. One might ask: Why do we persist in eating degerminated cereals merely because they keep better in that form? Why do we not solve the problems of rancidity and then eat the grains in their unaltered state? Is it not strange to put so much effort into a "bread-enrichment" program, worthy as it is, merely to *restore* to our daily diet some of the thiamine, niacin, riboflavin, and iron which has been lost in the manufacturing processes? Why should we go to all this trouble to solve a problem created by our own ineptitude? And even yet the problem remains unsolved for those persons of low purchasing power who must subsist to a considerable degree upon breadstuffs. This is so because the proteins of the cereal grains are naturally somewhat low in lysine, and this lysine deficiency is even greater in white flour and degerminated corn meal, thus limiting further their nutritive potentialities. Despite the many persuasive efforts to justify white bread nutritionally, the fact remains that lysine is an indispensable amino acid for man, essential for many of his metabolic needs. Because of this unquestioned fact the problem of white bread versus brown bread will not have been solved practically until it has been solved theoretically; and neither argument nor advertising can alter this situation.

A second regrettable aspect of our nutritional carelessness is typified by our tolerating the practice of overprocessing some of our grainstuffs. When in times of plenty we divert milk powder and soy protein to the manufacture of paints and glue, objection to the practice must be largely theoretical; but when foods sold for human consumption are overprocessed in such a way as to destroy both vitamins and essential amino acids, the procedure should be looked upon as a nutritional misdemeanor, correctible, if necessary, by law. For example, when a breakfast cereal whose lysine content or availability has been reduced by overprocessing so as to make it nutritionally inadequate, is sold with the suggestion that milk will supplement it adequately, the purchaser is actually being urged to buy a supplementary "tie-in" commodity in order to ensure the presence in the breakfast food as eaten of all the essential amino acids. Moreover, if the purchaser prefers to eat his breakfast food with fruit instead of milk, much of its protein value is lost. Ordinarily we justify these dietary customs by saying that we must be practical; and it is true that they do not represent a serious threat to child welfare because, among other things, the families most vulnerable, viz., those of low purchasing power, usually cannot afford to buy such expensive breakfast foods. Nonetheless, the fact remains that until we recognize the bad logic we are not likely to correct the bad practice.

Another example might be given in relation to animal feeding. Because the farmer is a practical man, he buys protein supplements for his growing pigs. In other words, he knows that pigs cannot grow well unless he adds sup-

plemental protein to their rations. But because he knows little about the importance of essential amino acids in the protein supplement he may fail to get full value for his money. Thus he may buy a bag of supplement labeled "Protein Content 53.4%." To make it more mystifying, the additional figures N x 6.25 may also be printed. What the farmer does not know is that much of the nitrogen in the supplement may be derived from connective tissue, a type of protein which is nutritively incomplete because of its low content of certain essential amino acids. Thus because of his lack of theoretical knowledge about essential amino acids in relation to protein utilization, the farmer, with all his practicality, may buy considerable amounts of relatively inert nitrogenous material. In all fairness it should be pointed out that the significance of these facts has not been appreciated until recently. Nonetheless the point again is that as long as the farmer insists upon being "practical" along these lines, he will continue to perpetuate error in his feed lot. As with white bread, a solution to the problem can be practical only when it is also theoretical.

These facts have become established only after years of cumulative research; but the accelerated progress of recent years stems largely from them. In the exploitation of these and other facts we must constantly strive to keep the proper relationship between causes and effects. For example, some years ago we heard a great deal about one-third of the nation being ill-fed. If this were true, the *fact* signified an urgent medical need for nutritional rehabilitation. In order to meet this need, however, most of the popular emphasis centered around a proposed installation

of a system of federally supervised medicine in order that all ill-fed persons could better meet their sickness costs, whether of the doctor or of the hospital. The question whether physicians in general were well enough informed about nutritional needs to solve the problem of malnutrition was largely minimized; moreover, little was said about the need for nutritional research as the keystone of practicality.

In this lecture I wish to point out some of the ways in which nutritional advances have come about in recent years, particularly in the field of protein nutrition. I do so because until recently the emphasis upon vitamins tended to overshadow the role of proteins in nutrition. But within the past decade the following discoveries have occurred in rapid succession:

1. Discovery of the complete series of so-called essential or indispensable amino acids.

2. Development of chemical and microbiological methods which now make possible the determination of the amino-acid composition of proteins and food-mixtures.

3. Development of methods of synthesis which have made available almost all of the dietary amino acids for experimental and practical use.

4. Demonstration of the mechanisms of plasma protein and hemoglobin formation and of the importance of the protein reserves. From these demonstrations has come a keener insight into the intricate problems of protein metabolism, including such aspects as transamination, transmethylation, the concept of the dynamic equilibrium, the synthesis of antibodies, wound healing, etc. The development of blood banks and plasma banks, so im-

portant both in the war and later, are practical outgrowths of these discoveries.

5. Further elucidation of several aspects of liver functioning, especially the roles of methionine, choline, and cystine in relation to cirrhosis, hepatitis, resistance to infection and intoxication, protein synthesis, etc.

6. Discovery and synthesis of several vitamins, notably thiamine, ascorbic acid, niacin, riboflavin, pyridoxine, pantothenic acid, folic acid, biotin and, most recently, vitamin B-12.

It is almost incredible that even ten years ago many of these facts were unknown. Now influential organizations, such as the Food and Nutrition Board of the National Research Council, are putting them into practical use as, for example, in the Bread Enrichment Program, and by means of the Dietary Allowance Tables set up to serve as aids to better nutrition. In retrospect we need but look at the record of the Selective Service Boards to realize that many young Americans were denied military status largely because of physical defects which may have been due, directly or indirectly, to poor nutrition.

In the remainder of this lecture I shall deal mainly with the problem of the essential amino acids and their relationships to dietary protein, and with the relationship of antibody mechanisms to the processes of acquired resistance to bacterial infection.

The Essential Amino Acids

The story of the essential amino acids covers a period of approximately three decades, eventuating in the brilliant discovery of threonine by Rose and his associates.

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Only after the isolation and synthesis of this amino acid was it possible to induce good growth in young rats fed rations whose dietary nitrogen came from a mixture of crystalline amino acids. Until then the studies of protein nutrition had to depend largely upon the estimation of crude nitrogen intake and outgo; in other words, upon the determination of nitrogen balances, together with arguments pro and con as to whether human beings were healthier on a high or a low protein diet. Now that the emphasis has shifted to the amino-acid make-up of proteins, and particularly, to their content of essential amino acids, progress has been amazingly rapid. Today we characterize a "complete" or "incomplete" protein in terms of its amino-acid composition; and within the past few years protein hydrolysates containing all the essential amino acids have become available for intravenous alimentation in surgical and medical practice.

These amino acids are daily dietary essentials because the mammalian body cannot synthesize them, at least in amounts adequate for most nutritional needs. To this extent they resemble vitamins, although they are needed in larger daily amounts. They differ from vitamins, however, in that they cannot be stored as much in the tissues and hence must be resupplied from day to day. Their discovery came largely from the now classic experiments of Osborne and Mendel. For example, in Mendel's monograph of 1923 we find the following statement:

Today we are concerned with the question whether this or that protein, whatever its biologic origin, will yield the characteristic desired amino-acids. Our attention is fixed on the building stones or units out of which the great protein structures are put together. Instead of referring to the proteins in terms of their physical properties or empirical composition—their content of

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carbon, hydrogen, oxygen, nitrogen or sulphur—at least so far as the problems of nutrition are involved, the time has arrived for estimating their behavior in the organism on the basis of the quota of each of about eighteen well-defined amino-acids·which the individual representatives of this group of foodstuffs can yield. Many, if not all, of these amino-acids are essential for the construction of tissue and the regeneration of cellular losses. In proportion as any specific protein can furnish these constructive units it may satisfy the nutritive needs of the body. The efficiency of the individual protein in this respect must depend on the minimum of any indispensable amino-acid that it will yield; for it is now known that some of them cannot be synthesized by the animal organism. If, for example, a protein or mixture of proteins comparatively deficient in their yield of the sulphur-containing amino-acid cystine be furnished alone to supply the body's nitrogenous requirements, the production of new, cystine-yielding molecules of protein will be limited by the amount which is available in the diet. An excess need not be wasted, for it can be burned up like sugar or fat to provide energy; but new construction or growth is limited by the minimum of the essential unit.

Thus at that early date we see expressed such currently familiar terms as "essential," "indispensable," "minimum," and "limiting" factor, ideas which today influence much of our thinking in this field.

During the past decade we have learned a great deal more about these essential amino acids. For example, we know now that the same eight are indispensable for the rat, the dog, the mouse, and man, whether measured by growth, by the maintenance of nitrogen balance, or for the formation of hemoglobin or plasma protein. The ninth one, histidine, although essential for the rat, the mouse, and the dog, has now been shown by Rose to be nonessential for the maintenance of nitrogen balance in man. This may mean merely that man has acquired the capacity to synthesize histidine, at least in adulthood, and can dispense with this particular amino acid in his food. However, for the other eight he still manifests the same

"synthetic disability" evidenced in the other three types of mammals.

Aside from the many physiological and pathological problems concerned with the utilization of these amino acids there are other ways in which they are of metabolic significance. Thus the nutritive potentiality of a protein or food mixture depends mainly upon its content of essential amino acids. With the development of newer methods for the amino-acid analysis of foodstuffs, both chemical and microbiological, it has become possible to determine fairly accurately the protein value of a foodstuff. In fact, owing to the operation of the "law of minimum" already alluded to, it can be shown that the varying potentialities of proteins depend essentially upon the absolute amounts and proportions of the essential amino acids in them or to their availability in the course of intestinal digestion. In consequence high-quality animal proteins are superior to those derived from cereals and legumes. Thus wheat is deficient in lysine, corn in lysine and tryptophane, peas and beans in methionine, etc. The recognition of these specific amino-acid deficiencies now makes it possible to effect nutritional improvement in certain foods by appropriate supplementation so that the amino acid-weaknesses of one food may be counterbalanced by proper blending with other foods richer in such amino acids. Thus such combinations as bread and milk, bread and meat, bread and cheese, corn and beans, all tend to provide amino-acid mixtures better adjusted for tissue development because of mutual supplementation of the dietary amino acids.

In the attempt to evaluate the nutritive potentialities of dietary proteins many methods have been tried. Of these the rat-growth method, and various modifications of the nitrogen-balance technique, have been most widely used. All have been criticized, however, either because of the time required for their performance, or because of their complicated nature. Nevertheless most that we know about protein values has been obtained in these ways.

During the war an urgent need arose for a rapid and comparatively simple method which might give a reasonably accurate measure of the protein values of rations and foodstuffs, particularly as they might have been modified by differing storage conditions and processing methods. Because of this need we developed a method which eliminates some of the disadvantages of the standard methods. This method we have called the Rat-Repletion Method. It consists in the use of adult male albino rats which have been made protein-deficient by a prolonged period of feeding of a ration adequate in calories, vitamins, and minerals, but low in protein. After the animals have lost approximately 25 per cent of their initial weights they are fed the depletion diet with its protein components replaced by the particular protein to be evaluated, added at approximately a 9 per cent protein level. This repletion ration is fed in equal daily amounts for periods of from 7 to 14 days and the resulting tissue repletion measured in various ways, i.e., in terms of weight recovery, regeneration of total plasma protein, hemoglobin, total carcass protein, as well as in other ways. Such animals respond quickly and manifest differences as influenced by the

quality of protein ingested. In fact, because much of the total amount of tissue regeneration is represented by the regeneration of muscle mass, the average weight recovery per animal gives a reasonably good measurement of the quality of the protein consumed. The repletion rations are made isocaloric and contain essentially the same amounts of vitamins and salts, or at least the minimal amounts which are presumably needed in the processes of effective tissue-protein synthesis. On such a repletion ration containing a high-quality protein, such as lactalbumin or an equal mixture of lactalbumin and casein, the depleted rats will, on the average, recover from 35-45 grams of lost weight in 7 days, from 50-55 grams in 10 days, and from 60-70 grams in 14 days.

Figures 1-6 demonstrate some of the ways in which this Rat-Repletion Method has been useful as a bioassay method for the evaluation of protein quality and for the
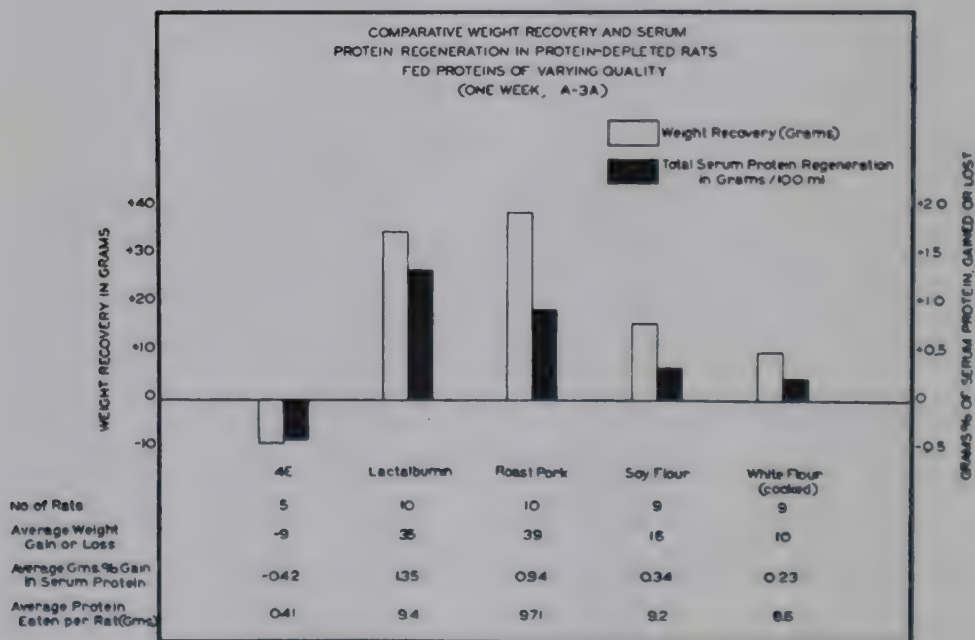

FIGURE 1

determination of amino-acid inadequacies in a particular protein.

For example, (figure 1) when groups of rats were fed rations containing four different proteins, viz., lactalbumin, roast pork, soy flour, and white flour, it is evident that in seven days the performance for soy protein was approximately 50 per cent of that for lactalbumin or roast pork, and for white flour, less than 30 per cent, despite the fact that the ingestion of "protein" was essentially the same in all instances. In view of the fact that the intake of calories, vitamins, and salts was approximately equal, this evidently represents the comparative nutritive potentialities of the dietary proteins tested. In short, amino-acid inadequacies in the vegetable proteins presumably acted as limiting factors.

Again (figure 2) when natural proteins and protein hydrolysates (enzymatically digested) were similarly compared, there was a good tissue repletion following the ingestion of such proteins as casein, fibrin, casein hydrolysate (amigen), fibrin hydrolysate, and skim-milk powder, whereas repletion following the ingestion of a soy protein water extract, and two hydrolysates prepared by acid hydrolysis, was negligible. It is obvious, therefore, that these latter foodstuffs manifest a poor capacity to engender the process of tissue-protein synthesis because of a lack in them of certain essential amino acids. With acid hydrolysis, for example, it is easy to demonstrate the loss of tryptophane, and much of the nutritive value of such a preparation can be restored by appropriate readdition of this amino acid. The point is, however, that the Rat-Repletion Method can quickly demonstrate such essential

amino-acid inadequacies under the conditions of the assay.

In figure 3 are shown the effects of amino-acid deficiencies in degerminated white corn meal. When this foodstuff was "enriched" by the addition of a complete vitamin and salt supplement and fed to a group of protein-depleted rats, the food consumption in the ensuing seven days was low and the weight gain negligible (an average of only about 4 grams per animal). Realizing the known amino-acid deficiencies of such a foodstuff, we added a mixture of eight essential amino acids to the corn meal and the corn meal was fed to the same group of rats for a second week. Immediately food consumption improved and the weight gains for the second seven-day period averaged 42 grams per rat. In short, the addition of eight essential amino acids had given the corn meal a protein potentiality essentially that of a high-quality animal
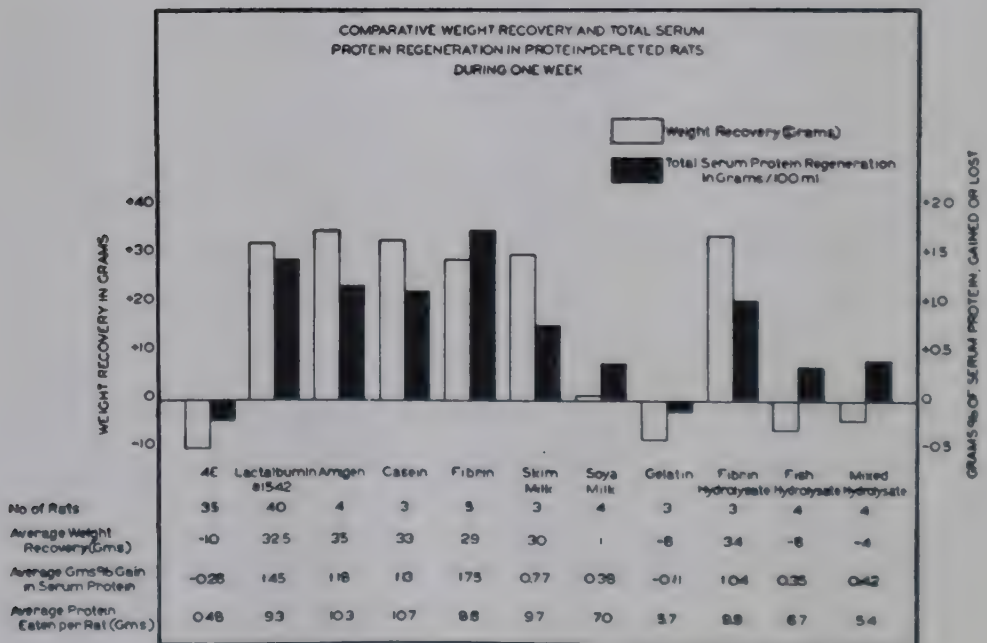

FIGURE 2

protein. Thus it is possible to convert a poor protein into a high-quality one merely by the addition to it of essential amino acids lacking or deficient in amounts in the poor protein.

Figure 4 shows the ability to improve the nutritive quality of a legume by appropriate amino-acid supplementation. When pea protein in a basal ration was fed to a group of protein-depleted rats for a period of fourteen days, the protein efficiency, expressed in terms of weight gain per gram of protein consumed, was approximately 40 per cent of that of a mixture of lactalbumin and casein similarly tested. Addition of small amounts of threonine
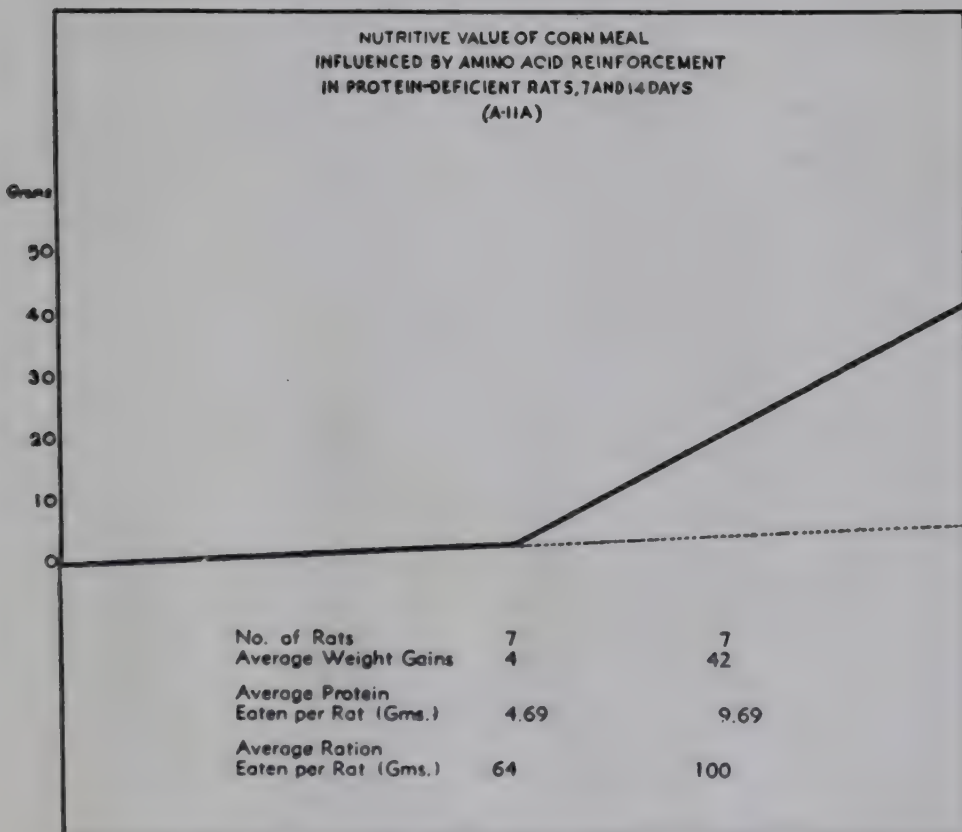

FIGURE 3*

*The corn meal was reinforced by addition of arginine, histidine, lysine, tryptophane, phenylalanine, threonine, methionine, valine, and isoleucine.

and methionine to the pea protein, however, improved the efficiency to approximately 65 per cent.

Further evidence that a relatively poor protein can be improved by appropriate protein or amino-acid supplementation is illustrated in figure 5. Thus, when white flour served as the sole protein in the repletion ration the performance in seven days was approximately 30 per cent of that of a high-quality protein (lactalbumin-casein) under similar circumstances. When, however, lysine, a lysine concentrate, or lactalbumin and skim milk were added to the white flour, performance was materially improved. On the other hand, when a flour which had been supplemented with approximately 20 per cent of lactal-

SUPPLEMENTAL ACTION OF ESSENTIAL AMINO ACIDS UPON NUTRITIVE VALUE OF PEA FLOUR   (14 DAYS) (A128-1)

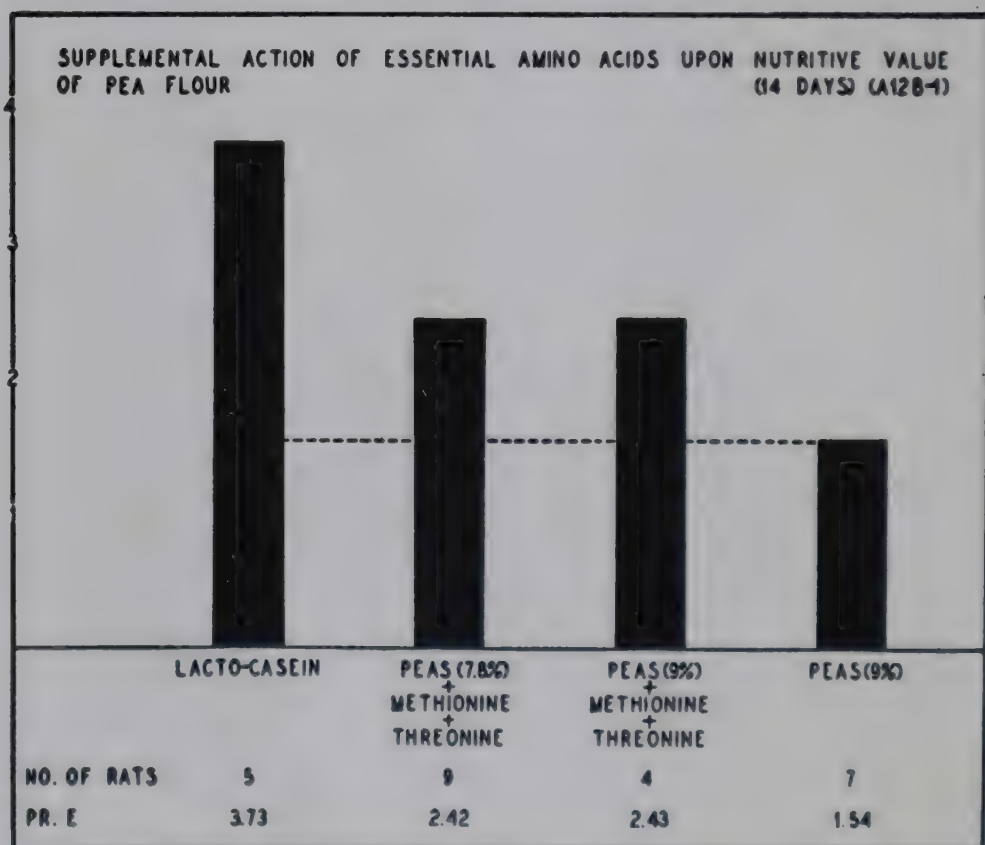|	LACTO-CASEIN	PEAS (7.8%) + METHIONINE + THREONINE	PEAS (9%) + METHIONINE + THREONINE	PEAS (9%)
NO. OF RATS	5	9	4	7
PR. E	3.73	2.42	2.43	1.54

FIGURE 4

bumin was heated at a toasting temperature until it be-
came a light brown, most of its protein value was lost, be-
cause the high temperature had made the lysine unavail-
able for intestinal digestion. Figure 6 demonstrates this
effect graphically, while showing, also, that when lysine is
readded to the heat-damaged mixture A, its former nutri-
tive potentiality is restored.

These charts are presented mainly to demonstrate
some of the ways in which the Rat-Repletion Method can
quickly reveal protein inadequacies in a food mixture,
whether due to natural defects, or as a consequence of
processing injury. Other examples may be found in our
published papers.

Protein Metabolism in Relation to Acquired Resistance to Bacterial Infection

Presumptive evidence that severe undernutrition en-
hances susceptibility to bacterial infection has long been
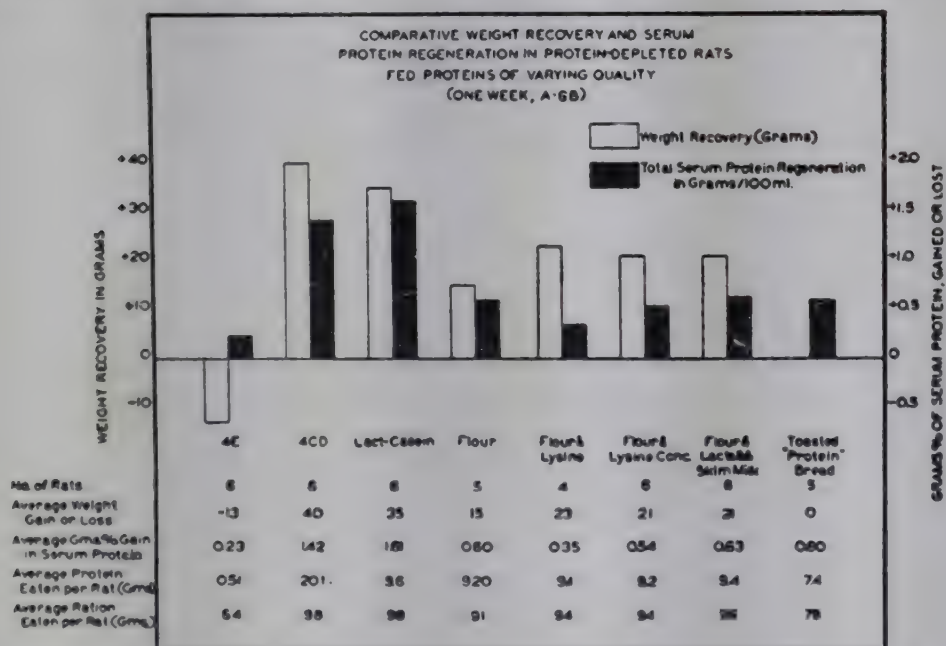

COMPARATIVE WEIGHT RECOVERY AND SERUM
PROTEIN REGENERATION IN PROTEIN-DEPLETED RATS
FED PROTEINS OF VARYING QUALITY
(ONE WEEK, A-68)

FIGURE 5

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Influence of Heating upon Protein & Effects of Lysine Supplementation
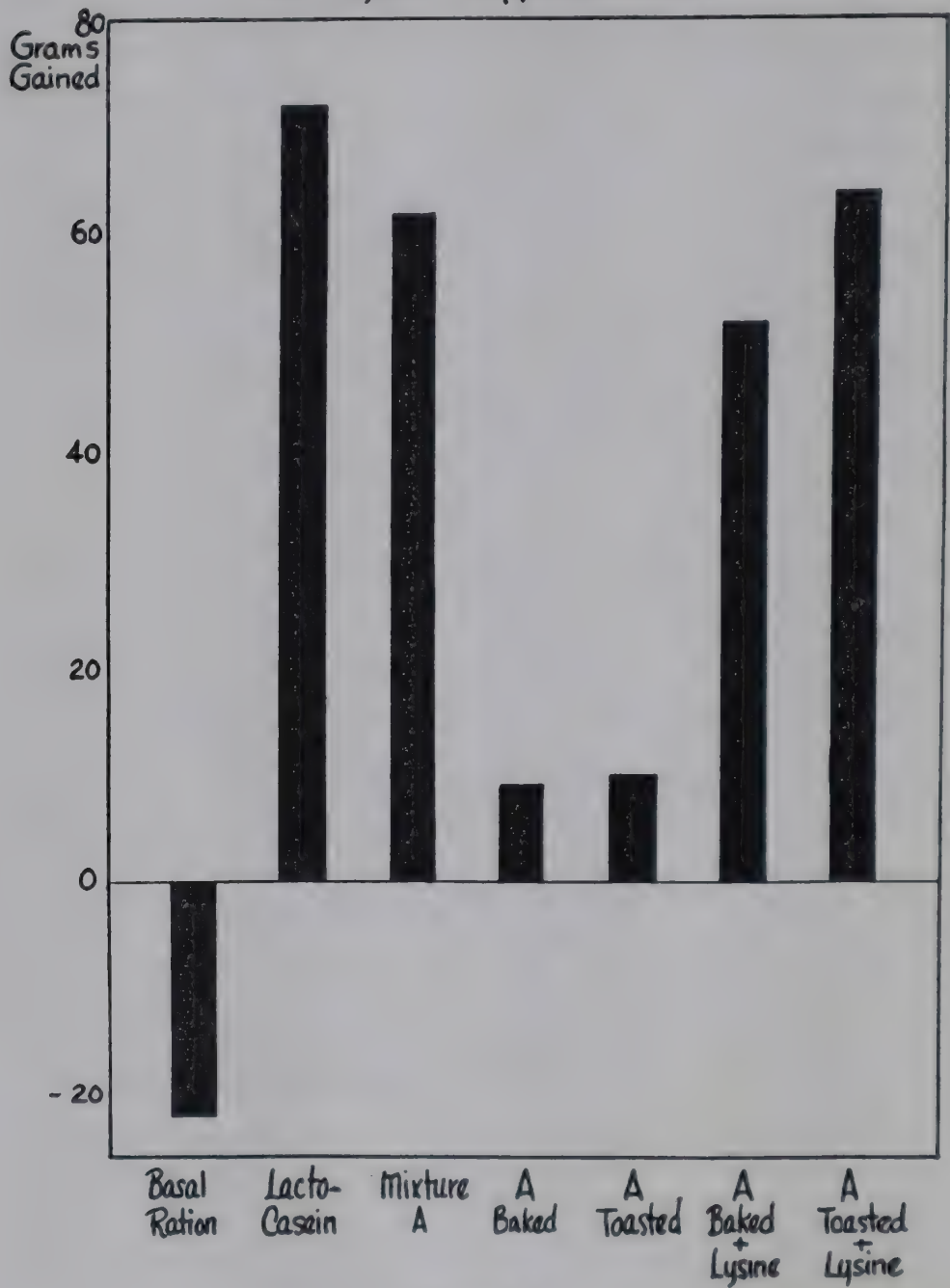
FIGURE 6

known, but experimental evidence supporting this supposition has been meager and inconclusive. Both types of evidence, however, although largely circumstantial, have been too suggestive to be lightly cast aside, particularly with respect to bacterial agents. By 1935 it had become apparent that the antibody molecule is a specifically modified molecule of plasma globulin. This important chemical fact opened the way to an experimental approach to the general problem of the relationship of protein metabolism to the acquisition and maintenance of acquired immunity. In short, if normal plasma globulin and antibody globulin are chemically identical, and if these blood constituents differ only in respect to the latter's affinity for antigen, their mechanisms of fabrication might well be similar. Moreover, after Brand et al. had revealed the amino-acid content of human gamma globulin and had demonstrated that it contains several of the essential amino acids, it became all the more likely that the fabrication of gamma globulin must be dependent ultimately upon an adequate dietary intake of all amino acids essential for the synthesis of this protein molecule.

From the experiments of Whipple, Madden, and their associates evidence was already available indicating that food may influence the fabrication of plasma proteins. They had also pointed out that both albumin and globulin synthesis is regulated by the character of the diet; and inasmuch as the nutritive potentiality of a dietary protein depends upon its amino-acid composition, it was logical to assume that the production of globulin must likewise be the consequence of amino-acid utilization in the pro-

cesses of protein synthesis. Further evidence for this postulate was supplied by experiments in which we demonstrated that when protein-depleted rats were fed a diet adequate in all dietary essentials, but with the dietary nitrogen derived from a mixture of crystalline amino acids, the removal singly of any one of the nine indispensable amino acids led quickly to a slowdown or stoppage of plasma protein fabrication. This indicated, therefore, the essentiality of each of the nine amino acids for the fabrication of the plasma protein molecule.

In order to put these ideas to further experimental tests we subjected rabbits and white rats to prolonged protein depletion in order to accomplish a marked reduction of their protein stores as evidenced by the production of a severe degree of hypoproteinemia and a marked loss of weight. When these animals were then subjected to antigenic stimulation they displayed a distinctly lessened capacity to elaborate specific antibody; moreover, after repletion with high-quality protein or even with a mixture of crystalline amino acids, their decreased capacity to fabricate antibodies quickly returned toward the normal. Furthermore, protein-depleted animals were found to be considerably more susceptible to induced infection, and also less immunizable than well-nourished ones. By bio-assay we also found that human gamma globulin contains all of the essential amino acids, suggesting, in consequence, that in the course of starvation it should be difficult for the body to manufacture such a complex protein as antibody globulin in the absence of an adequate supply of essential amino acids (figure 7). We suggested, therefore, in a series of publications, that the increased suscept-

ibility to infection displayed by starving persons may be best attributed to depletion of their protein reserves and their inability to fabricate new supplies of antibody globulin because of an inadequate intake of essential amino acids.
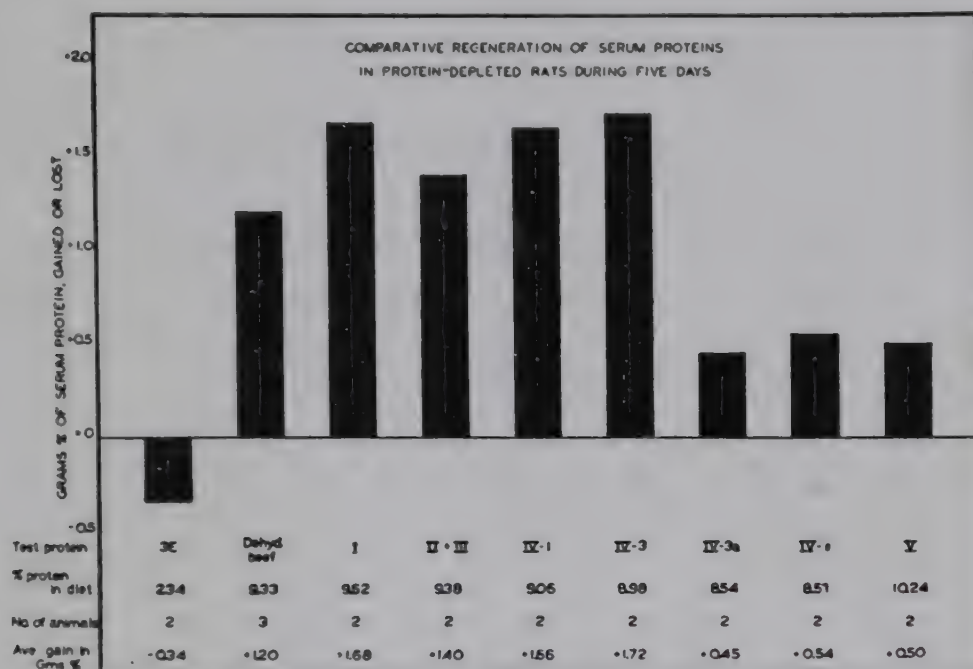

FIGURE 7*

Dietary Essentials in Relation to Tissue-Protein Synthesis

II

Dietary Essentials in Relation to Tissue-Protein Synthesis

NUTRITIONAL DEFICIENCY disease develops primarily because of a dietary lack or insufficiency of one or more essential nutrients. These nutrients are essential because the body cannot make them and must get them from the daily food. They include a variety of amino acids, vitamins, salts, fatty acids, and even particular chemical groups, and comprise a total of at least forty different groups or substances.

If the treatment of malnutrition depended solely upon the dietary absence of one or more of these essential nutrients the therapeutic problem would be a simple one of specific replacement therapy. There are situations, however, where, even in the presence of all of these indispensable dietary constituents, abnormal bodily states tend to interfere with their proper utilization, as, for example, because of an interference with digestion, absorption or assimilation, an increase in metabolic needs, or an accelerated rate of destruction or excretion. Because of such modifying or "conditioning" factors the diagnosis and treatment of nutritional deficiency may at times become exceedingly difficult. Thus obstructive or ulcerative lesions of the digestive tract may impair appetite or interfere with the mastication, digestion, or absorption of food; hypermotility of the gastrointestinal tract may lessen food

absorption; achlorhydria may hamper digestion; and infectious or metabolic disease may interfere with effective food utilization. But whether nutritional disease results from primary or secondary causes, ultimately it manifests itself as a tissue-deficit; and one must examine the tissues for evidences of the deficiency.

The signs and symptoms of some types of malnutrition may be definite enough to characterize them as specific, as, for example, the hypochromic anemia of iron deficiency, the thyroid enlargement of iodine deficiency, or the bleeding tendency of ascorbic-acid deficiency. With protein deficiency, however, no such clear-cut manifestations are ordinarily seen. On the contrary a variety of signs and symptoms may develop concomitantly. These may include pallor, weakness, weight-loss, anemia, hypoproteinemia, edema, slow pulse, low blood pressure, hypothermia, anorexia, and polyuria, none appearing singly or predominantly. Moreover, because protein deficiency may be accompanied by both vitamin and calorie deficiencies, one cannot always establish the primary cause of a particular set of symptoms. Nevertheless, excepting for the loss of fat, tissue loss ordinarily means a loss of tissue-protein, and the therapeutic problem centers around the reasons for the loss and the remedial measures called for in order to accomplish tissue repletion. In short, the process of convalescence is essentially a nutritional problem of tissue synthesis, and it includes such varied syntheses as the regeneration of muscle mass, of blood and tissue proteins, of red blood cells, leukocytes, and certain hormones and enzyme systems.

Because a debilitated patient has lost such a large proportion of his tissue reserves, including protein, carbohydrate, fat, vitamins, and salts, the depleted tissue stores must be repleted. The problem is: What kind of diet will best restore them to their original state?

Obviously a first requirement is the discovery and removal of the cause or causes of the deficiency. If the cause is primary, an ample intake of the missing dietary essentials should suffice. If, on the other hand, the cause is secondary, the therapeutic problem is more complex; and it would be unreasonable to expect an adequate repletion of depleted reserves in the presence of an interfering factor. Under such circumstances elimination of the conditioning factor becomes the primary problem and the deficiency itself is a secondary consideration. Consequently, if conditioning factors restrict the effective utilization of dietary essentials even though all essential nutrients are present in the daily food, convalescence may be slow. For example, protein synthesis may be lessened in the presence of hepatic disease, nephritis, or infection, despite the ingestion of adequate amounts of nutritious food. Support for this assertion is seen from experiments of Whipple and his associates in which plasma protein fabrication was markedly impaired in the course of infection or after the production of sterile abscesses. Nevertheless, physicians too often forget or are unaware of these facts and become discouraged at the slowness of convalescence in relation to specific dietary regimes.

Because we usually think of nutritional "limiting factors" in terms of essential amino acids we are prone to forget that either the dietary absence or the inadequate

assimilation of any essential nutrient constitutes a limiting factor and thus leads to a tissue deficit. Therefore, in the search for an optimal diet one should assume that it must be completely adequate, both in composition and in utilization, if it is to be optimally effective in tissue synthesis; and surely nothing less is desirable for the alimentation of a seriously ill patient.

In evaluating the nutritive importance of the various essential nutrients calories obviously must be accorded a primary role, representing as they do the thermal units which supply energy for every metabolic need. It should be borne in mind, however, that in a high-calorie diet it is not just calories, but the overall food intake, which makes the diet effective. For example, a diet composed of sugar, lard, starch, alcohol, and an abundance of vitamins would contain an adequate supply of calories and still carry little nutritive value. As Cathcart said many years ago:

> We do not live on calories . . . . Calorie value is simply a very convenient physical standard for the assessment of diets, but merely because such a standard has proved of great utilitarian value there is no real justification for placing this standard as the foundation stone of hypotheses framed to offer an explanation of cellular activity. Many writers are obsessed with the idea of the calorie, forgetting that the organism is certainly not a heat engine. It is perfectly true that calories are a measure of heat, but it must not be forgotten that we do not consume actual heat units but only potential heat-giving substances which can eventually be degraded to the form of heat and be measured as such. The thermal aspect of nutrition is unduly stressed, for, while heat may be a necessary product of tissue activity, it is, after all, a by-product.

In other words, the living organism operates both to release food-energy and to build and maintain tissues from the same materials; and when it undergoes structural injury or deterioration its functions become corres-

pondingly impaired. In consequence, a vicious circle may be initiated which further hampers repair of the organism itself. Thus the processes of growth, maintenance, and repair require not only calories but amino acids; for these constructive units must enter into the protein structure of the living machine, under the activating direction of calories, vitamins, and salts.

On the other hand, the basic importance of calories should not be underemphasized nor can the fact be minimized that a subcaloric diet cannot function optimally. For basal needs the body obviously requires enough calories to maintain itself; otherwise it will utilize its available stores of carbohydrates, fat, and tissue protein. Here, too, a calorie-deficiency acts as a limiting factor, whether for growth or maintenance. This limiting effect was often seen in the days when physicians intentionally underfed febrile patients in accordance with the old doctrine of feeding a cold and starving a fever. Because of this erroneous notion patients tended to lose large amounts of tissue-nitrogen in the course of infectious diseases; and not until the advent of the Coleman-Shaffer high-calorie diet in the treatment of typhoid fever did it become apparent that much of the tissue-wastage was needless and preventable. At about that time arose interest in the problem of the so-called "toxic destruction of protein," a problem which, even now, remains unsolved. For example, we do not know yet whether the loss of tissue results from the toxic action of bacteria, from the lack of calories, vitamins, minerals, or amino acids, or from a combination of these or other factors.

In an effort to obtain more insight into the role of calories in relation to protein utilization we have reported experiments designed to ascertain the extent of tissue protein synthesis under standardized conditions in protein-depleted adult white rats. As was mentioned in the first lecture, such animals respond quickly to a good repletion diet and demonstrate thereby the effects of various dietary factors upon convalescence. Our standard repletion ration supplies 48 calories per rat per day, 1.35 grams of high-quality protein, and an adequate content of vitamins and salts. By readjusting this diet so as to supply calories in varying amounts it was possible to demonstrate the differing effects of caloric intake upon the regeneration of depleted tissues. For example, several rations were fed in which the intake of protein, vitamins, and salts was kept constant, whereas the daily caloric intake varied from 15 calories per rat to 60 calories (figure 8). The results showed that at and above a caloric intake of 35 calories per rat per day, weight recovery was rapid; and by carcass analyses it was possible to demonstrate that above a level of 35 calories per rat per day the extent of protein synthesis did not greatly vary. In other words, an increase in caloric intake above a level of 35 calories per rat per day did not increase the utilization of dietary protein but led, rather, to the laying down of extra fat (see Benditt et al., 1948). On the other hand, when the animals were fed rations containing less than 35 calories per rat per day, weight recovery was slow despite the daily intake of 1.35 grams of high-quality protein. These effects reëmphasize the well-known fact that protein utilization requires an adequate caloric intake, and that this is hampered by sub-

caloric intakes. They also call to mind the difficulty in securing good utilization of protein hydrolysates by intravenous use in patients on a subcaloric diet. It is to be hoped that the efforts to prepare fat emulsions for intravenous administration may soon make possible an adequate caloric intake, thus ensuring the more effective utilization of injected amino-acid solutions.

But calories alone do not build tissues; the latter must be built from proteins by the utilization of their amino acids in adequate amounts, and particularly without those which the body cannot synthesize, tissue synthesis must inevitably slow down or stop. Here, too, experiments with

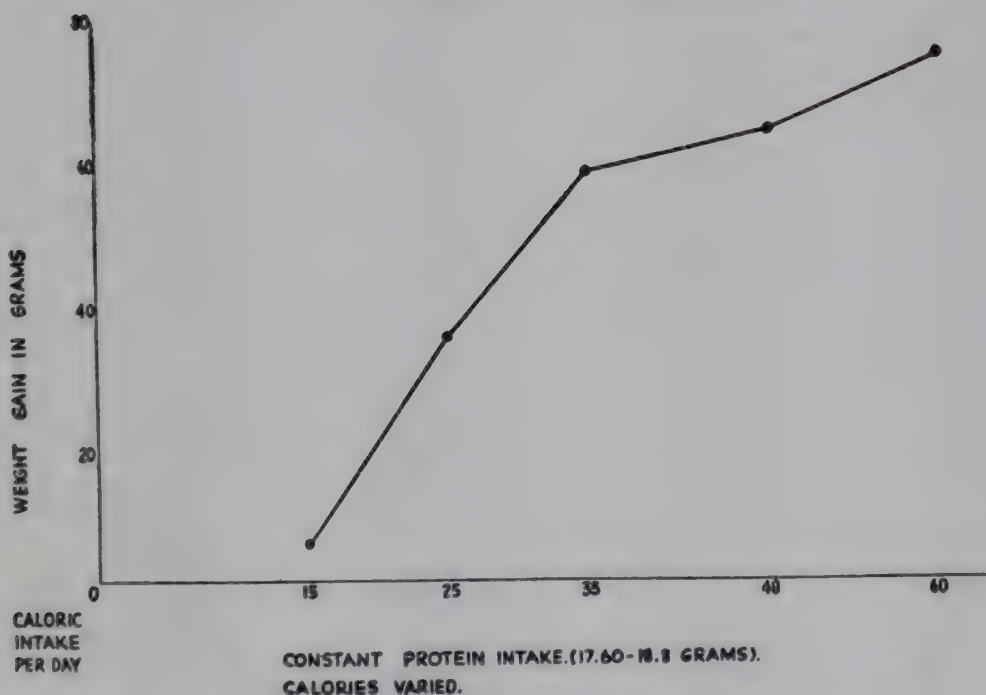## THE INFLUENCE OF CALORIC INTAKE UPON WEIGHT RECOVERY. (14 DAYS)

FIGURE 8*

*Each point on the curve represents the average performance of five rats fed the repletion-ration for fourteen days.

protein-depleted rats helped to elucidate the problem further. For example, when the rats were fed our standard high-calorie ration of 48 calories per rat per day, but containing decreased amounts of high-quality protein, convalescence proceeded in direct relation, not with the caloric value, for this was always the same, but with the protein content (figure 9). With smaller amounts of protein, weight recovery was negligible despite an ample intake of calories. Thus whether for effective recovery of lost weight or for the regeneration of plasma protein and hemoglobin these experiments demonstrated (1) the mutual indispensability of two dietary essentials, high-quality protein and calories, and (2) the fact that a deficiency of either
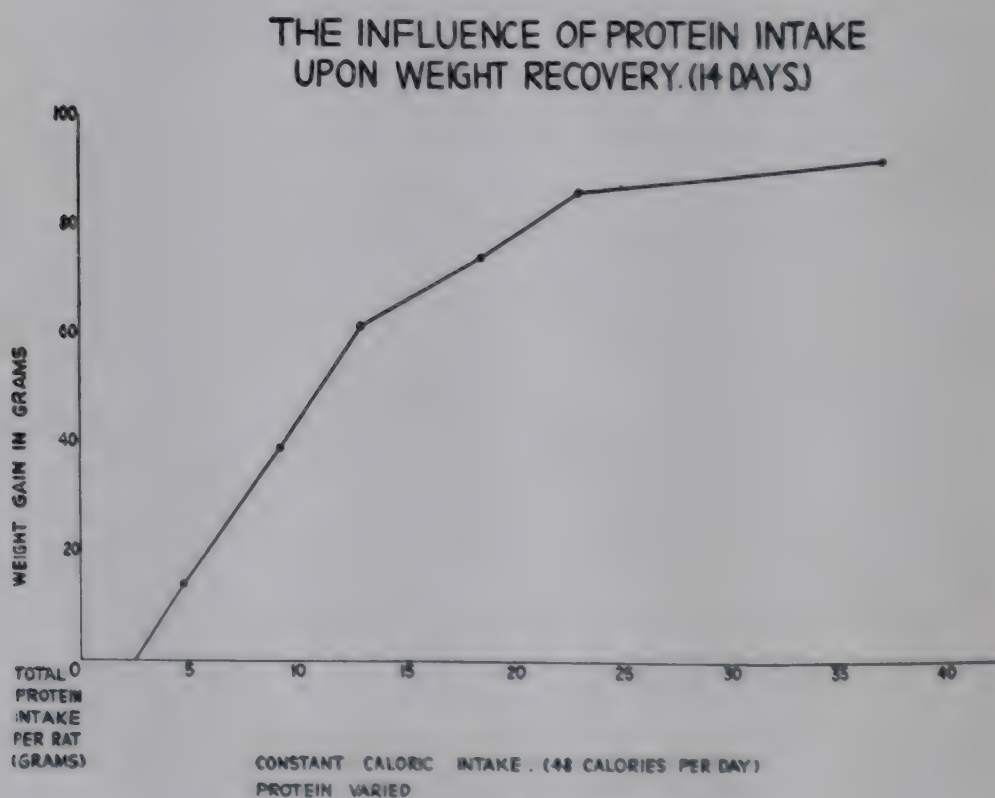
THE INFLUENCE OF PROTEIN INTAKE
UPON WEIGHT RECOVERY. (14 DAYS)

FIGURE 9*

*Each point represents the average performance of a group of five rats fed the repletion-ration for fourteen days.

calories or protein may constitute a limiting factor which hampers effective convalescence.

We wished next to ascertain the influences of vitamins upon the general processes of tissue-protein synthesis, for it has long been evident that such primary food constituents as protein, carbohydrate, fat, and salts cannot by themselves effect good nutrition, even in the presence of an adequate intake of calories.

In studying the role of the vitamins in protein synthesis a difficulty has been the elimination of all traces of vitamins from the ration. Fortunately, however, this diffi-
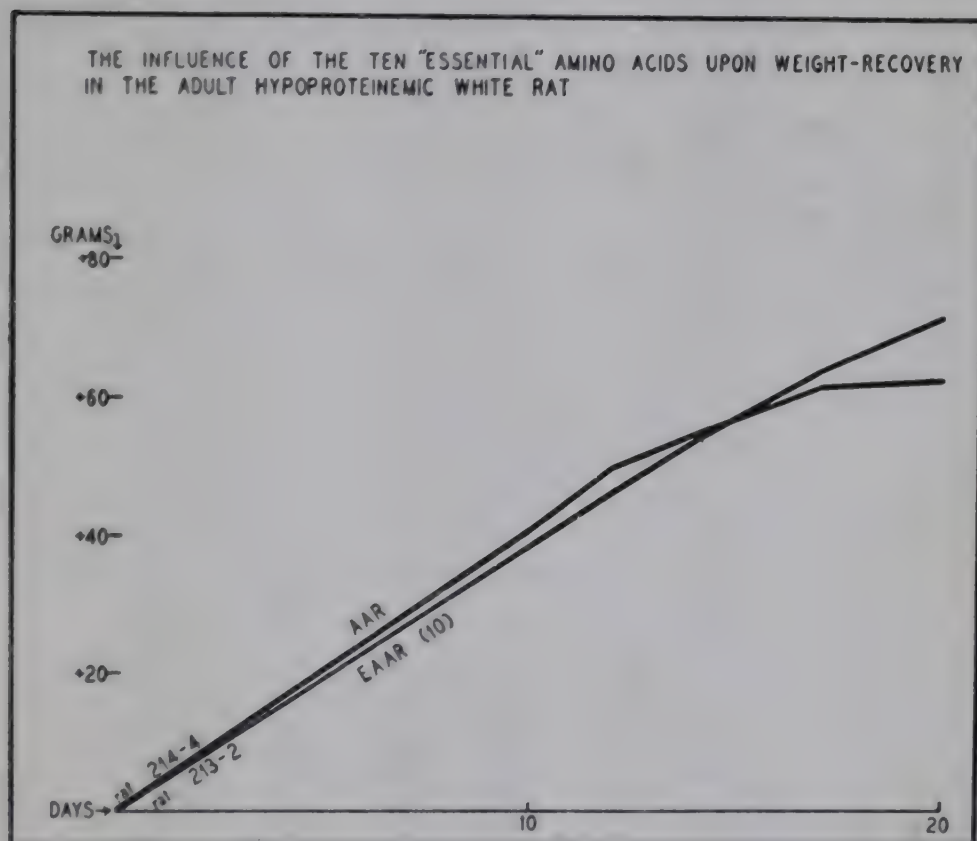
THE INFLUENCE OF THE TEN "ESSENTIAL" AMINO ACIDS UPON WEIGHT-RECOVERY IN THE ADULT HYPOPROTEINEMIC WHITE RAT

FIGURE 10*

*The two rats were fed the same basal ration in which the dietary nitrogen came either from a mixture of sixteen crystalline amino acids (AAR) or the ten "essential" amino acids (EAAR).

culty can now be obviated by the use of amino-acid mixtures as substitutes for natural protein, and it is now possible to make a mixture of sixteen crystalline amino acids, compounded in the proportions in which these occur in casein, and substituted in a basal ration adequate in other respects. Thus we have shown by our Rat-Repletion Method that animals eating such a ration will make practically as rapid weight gains as animals fed a natural protein (figure 10). This fact makes possible the study of the influence of various vitamins upon the processes of tissue protein synthesis. For example, when such a ration was fed to a group of protein-depleted rats for a period of 10 days the weight recovery averaged between 40 and 50 grams per rat. If, on the other hand, all of the B-complex vitamins were removed from the ration the animals in ten days made little if any weight gain. Then, by removing one vitamin at a time it was possible to evaluate the comparative role of any one vitamin in the overall process of
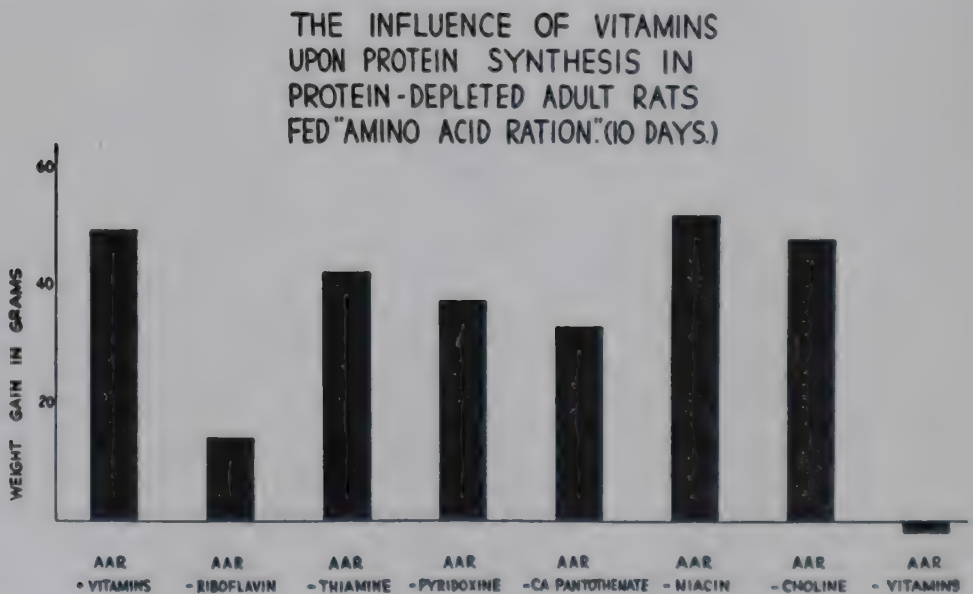

FIGURE 11

tissue protein synthesis. Under such circumstances the removal singly of niacin or choline had but little effect, whereas a lack of riboflavin particularly led to a partial block in the process of protein synthesis (figure 11).

These findings are not surprising in view of some earlier ones in which we had found that rats fed rations devoid of vitamins exhibited a marked reduction in their capacity to form specific antibody, comparable, in fact, to that found in chronic protein deficiency. These animals also lost weight and serum protein comparably, suggesting that a severe vitamin deficiency might be reflected in a retardation of the processes of protein synthesis. Thus the lack of response in the absence of riboflavin could be explained. In these experiments it was noted that the first manifestation of an interference with weight recovery in riboflavin deficiency came at the sixth day. This suggested that the livers of the depleted rats might be low in riboflavin stores; and analyses for riboflavin showed that this was so. We infer from this fact that a deficiency of riboflavin may hamper the construction of certain tissue proteins, particularly the more stable ones, as, for example, those of muscle tissue. It may be that the relatively high content of riboflavin in many types of high-quality protein, including meats of various kinds, may favor the more efficient synthesis of tissue protein in the living animal. Whether the riboflavin functions mainly in relation to cell respiration or in other ways is still unknown. But at any rate these types of experiments serve to reëmphasize the closely integrated metabolic relationships between protein, vitamins, fat, and carbohydrate, both in the construction and maintenance of tissues.

From what has been said thus far it is evident that the synthesizing mechanisms operate effectively only under the following circumstances: (1) They must have available all essential dietary constituents; (2) the synthesizing cells must be uninjured in order that they may obtain the energy necessary for the task of synthesis; and (3) conditions must be such that these cells can do an effective job of synthesis once they have initiated the process.

Although comparatively little is known about the enzyme systems concerned in protein synthesis it is generally assumed that certain salts and vitamins are necessary adjuncts in the conversion of amino acids into peptides and tissue proteins. When, moreover, the mechanism starts to function the process is, in all likelihood, rapid and "all or none" in character. More than twenty years ago W. C. Rose called attention to "the fundamental and irrefutable fact that the animal organism is unerringly accurate in its syntheses. If a tissue is to be formed at all every component required must be available or capable of being manufactured by the cells; otherwise the synthesis will not occur. If growth follows the addition of an essential constituent to an inadequate diet, it does so because cell reactions which could not proceed in the absence of the added factor are now made possible."

Although the reconstruction of depleted tissues requires not only adequate calories as well as a proper assortment and amounts of vitamins and proteins, it also requires a proper assortment and proper amounts of each of the indispensable amino acids. For example, in our experiments in which a mixture of sixteen crystalline amino acids was substituted for casein in the basal ration,
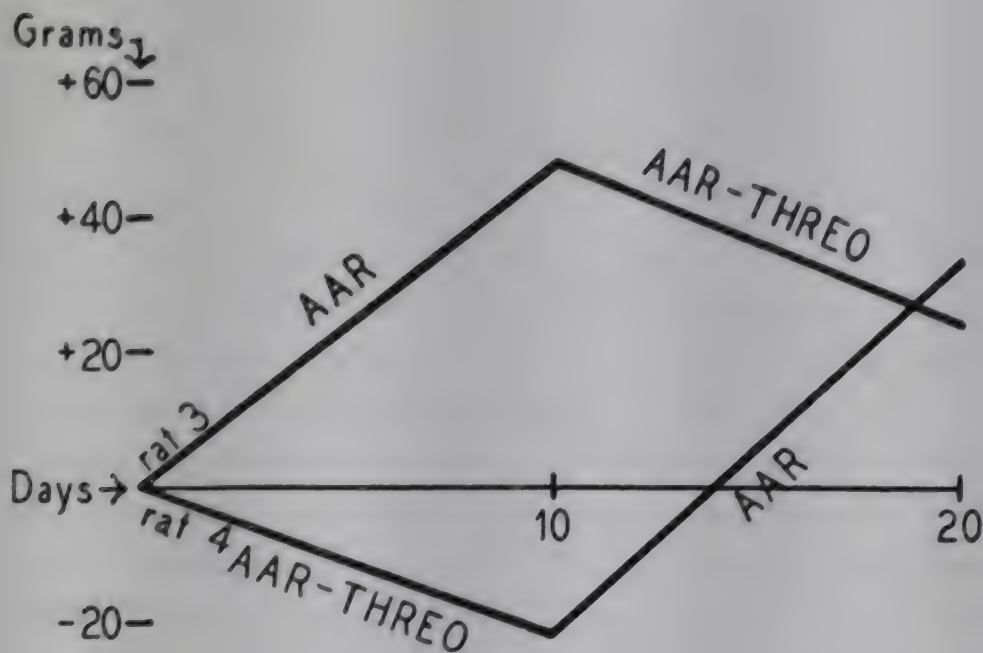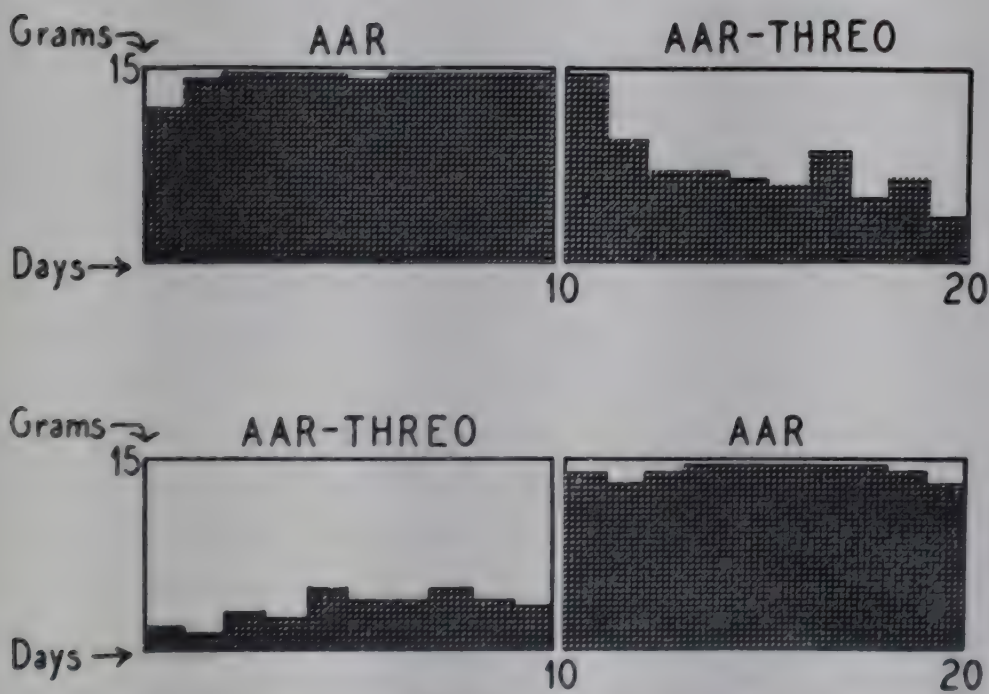
FIGURE 12

FIGURE 13

the rats regained lost weight practically as well as if they had been fed the natural protein. If, however, any one of nine indispensable amino acids was omitted from the ration the animal quickly lost appetite and not only failed to regain lost weight but actually continued to lose weight (figures 12 and 13). In fact, if any one of the nine indispensable amino acids was fed in an amount lower than its minimal daily requirement, even though all the rest were present in the diet, the animal failed to thrive. In other words, these indispensable amino acids are absolutely essential for the maintenance of appetite and for the rebuilding of depleted tissues, and they are essential every day and in definite amounts and proportions. They are, therefore, both dietary indispensables and dietary expendables, and must be replaced from day to day. This fact indicates that, despite the evidence of the importance of the tissue-protein stores, as will be emphasized in the third lecture, in enabling the body to meet certain emergency situations in the course of protein metabolism, there seems to be no corresponding tissue reserve of individual essential amino acids, at least in amounts available for free interchange in time of urgent need. The phenomena of loss of weight, of tissue nitrogen, and of appetite in the rat, the dog, and man appear so quickly when the diet lacks only a single essential amino acid that it seems difficult to believe that any amino-acid reserves are available. Moreover, the individual comes quickly back into nitrogen equilibrium when the missing dietary essential is restored to the diet. Evidently, then, not enough of the essential amino acids can be extracted from the tissues to compensate for their dietary absence; and

any accessory synthesizing mechanisms, as, for example, the intestinal bacteria, cannot fabricate the missing essentials rapidly enough to compensate for their dietary absence. That this does not apply only to protein-depleted rats is shown by the further fact that adult, well-nourished rats also lose weight and appetite when fed rations adequate in calories, vitamins, and salts and containing all essential amino-acids except one (figure 14). In other words, they, too, manifest acute amino-acid deficiency disease and are unable to correct the deficiency by the "raiding" of other tissues for a supply of essential amino acids. In the absence of a single essential amino acid, therefore, tissue synthesis comes to a standstill, as evidenced by the loss of weight in a well-nourished animal and by the inability of a depleted one to regain lost weight or to fabricate plasma protein or hemoglobin. In consequence all the other amino acids become useless for many purposes of protein synthesis, and, unless they can fit into some protein pattern, are presumably either converted into glucose or are deaminated and excreted. In short, the
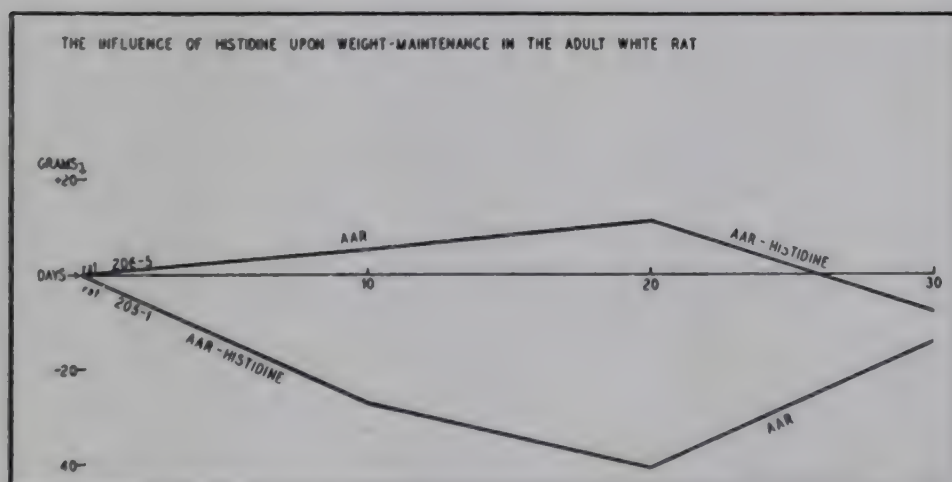

FIGURE 14

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synthesizing mechanisms apparently discard all essential amino-acid building stones under these altered circumstances and wait until a complete assortment is available before again starting the synthesizing process.

Further support for this concept is supplied by experiments in which the essential amino acids were fed together or in separate groups in an attempt to ascertain whether they must be simultaneously available to the synthesizing mechanisms for effective tissue utilization. As has been mentioned above (see figure 10), it is possible to secure good muscle regeneration by the feeding of a ration containing only the ten essential amino acids. Under such conditions the mixture is ingested simultaneously and the amino acids, presumably, are simultaneously absorbed. Thus when they reach the liver and other tissues they become immediately available *as a group* to the synthesizing mechanisms for the construction of tissue protein of various kinds.

We wished to determine, however, what would happen if these same essential amino acids were not made simultaneously available to the synthesizing mechanisms. In this way one could test critically the postulate that the essential amino acids must be simultaneously available to the synthesizing mechanisms in order to be built up into complete tissue proteins. We did this in the following way: A ration was compounded whose dietary nitrogen came from a mixture of the ten essential amino acids. Two other rations were similarly prepared except that one contained only five essential amino acids, viz., arginine, histidine, leucine, lysine, and threonine, and the other, the second five, viz., isoleucine, methionine, phenylalan-

ine, tryptophane, and valine. Each mixture of five amino acids had an essentially identical nitrogen content and the rations could be fed either simultaneously or alternately, and at intervals. Thus, when the two incomplete amino-acid rations were mixed in equal proportions and were fed to protein-depleted rats, the animals ate well and recovered lost weight steadily. If, moreover, they were fed at alternating intervals, from one to eight hours apart, the rats accepted the rations well and convalesced rapidly. On the other hand, if they were fed the incomplete rations under similar conditions they quickly stopped eating and lost weight rapidly. This even happened when the two incomplete amino-acid rations were alternated at hourly intervals. In fact, if the two incomplete rations were placed in a single cage and the complete ration in another cage and both groups of rats were allowed to eat *ad libitum*, those offered the complete ration gained weight steadily, whereas those offered the incomplete rations either lost weight or were barely able to maintain it (figures 15, 16, and 17).

It would seem from these experiments that an animal cannot eat one set of rations quickly enough in relation to the other to enable the second group of essential amino acids to combine with those of the first group in the synthesizing mechanisms in order to accomplish tissue synthesis. These experiments present challenging evidence, therefore, that for effective tissue synthesis all of the essential amino acids must be available at approximately the same time. Evidently, when one group of five amino acids has been absorbed, the individual amino acids are not stored in the liver or elsewhere for even one hour in

FIGURE 15

44

FIGURE 16

order to couple with the second group and thus be built up into protein molecules.

These facts tend to corroborate the hypothesis that the processes of tissue synthesis must be extraordinarily rapid. They suggest, moreover, that for optimal utilization of amino acids in tissue synthesis each meal should

Amino Acid Utilization
In Relation to Time
of Ingestion
(2 hr. alternation)
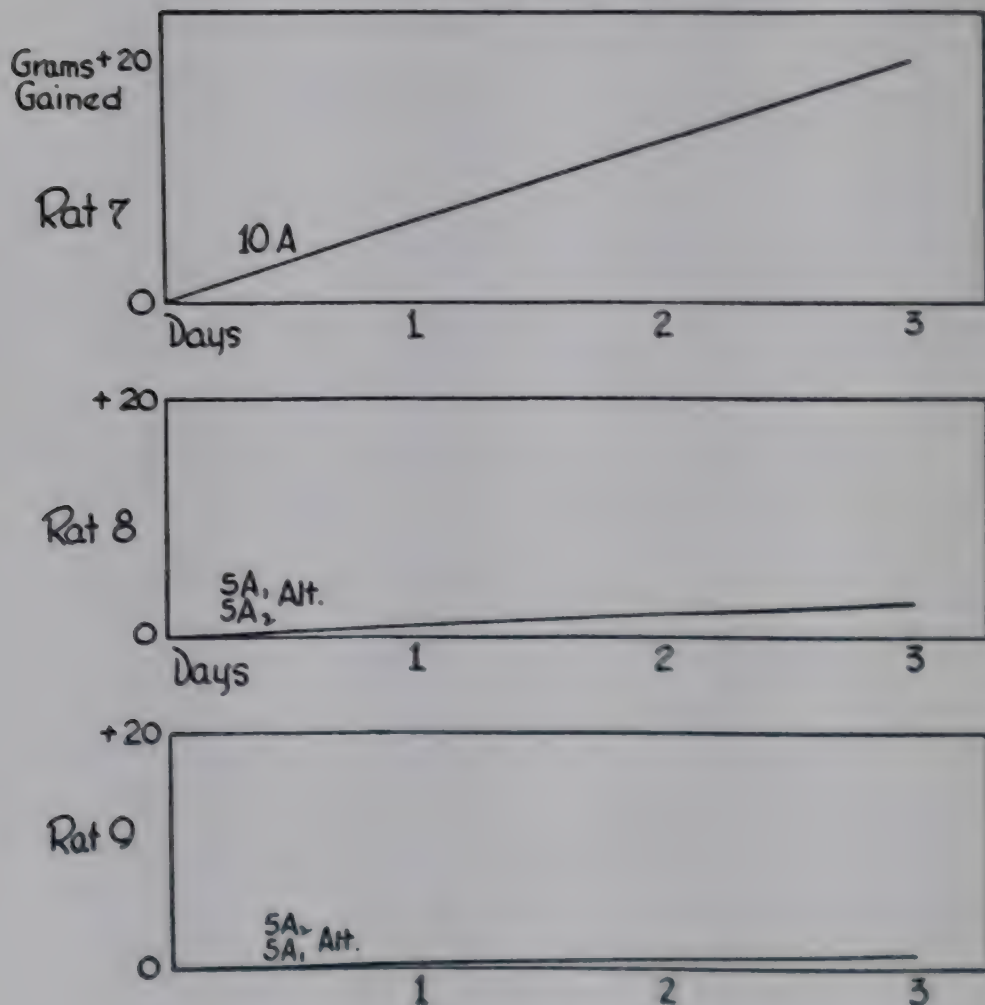
FIGURE 17

be well balanced with respect to its content and relative proportions of essential amino acids. It has already been suggested by Melnick and his associates that the unequal rate of enzymic digestion of some vegetable proteins, as for example, soy protein, with the resulting unequal rate of absorption of the essential amino acids resulting from this digestion, might well explain the fact that these proteins have a generally poorer nutritive value than do the animal proteins. Our experiments would tend to substantiate this postulate. Moreover, our findings reëmphasize the importance of the high-quality proteins, namely those in meat, milk, fish, and eggs, in tissue synthesis, both because of their easy digestibility and because of their rich content, and, in the right proportions, of all the essential amino acids.

Pathologic Aspects of Protein Nutrition and Their Relationship to Amino-Acid Utilization

III

Pathologic Aspects of Protein Nutrition and Their Relationship to Amino-Acid Utilization

IN THE preceding lecture the role of "conditioning factors" was considered from the viewpoint of their influence upon anabolic processes of protein metabolism. The conclusion was drawn that tissues can grow and maintain themselves, and effectively counteract various pathic stimuli, only if they are supplied with continuous and adequate amounts of dietary protein. In this lecture attention will be directed mainly to some of the catabolic aspects of metabolism relating to amino-acid utilization, and to certain therapeutic problems related to them.

According to the postulate of Whipple and his associates the mammalian body normally contains a reserve supply of tissue protein which is in dynamic equilibrium with circulating protein. Because of this equilibrium there exists a so-called "steady state," a sort of "protein give and take." So long as there is a continuous and adequate dietary supply of good-quality protein, amino acids released during digestion, and possibly small peptide groups as well, are absorbed and synthesized in the liver and elsewhere into protein aggregates which become available for the body's metabolic needs. In this concept the liver is pictured as the "key" organ in the process of protein
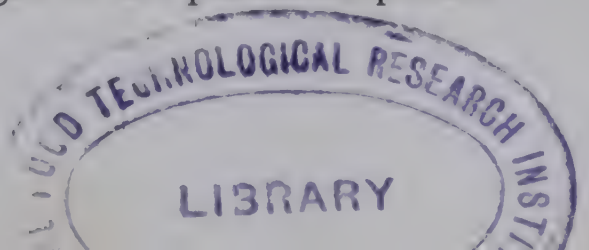
synthesis; and from the liver protein-material goes into plasma protein, hemoglobin, muscle, and other tissues. The total aggregate of protein constitutes the body's "protein pool" and serves as a sort of overall nutrient to satisfy the continuing metabolic needs.

The question whether individual cells can utilize this protein directly, however, is still unsettled. Although Whipple suggests this as a likely mechanism, he does not eliminate the possibility that protein molecules or aggregates may first undergo degradation into amino acids before being incorporated into cellular constituents. In any case it is now evident that homologous plasma can serve as the sole exogenous source of dietary protein, provided that an otherwise adequate diet is also eaten. On the other hand, a puzzling fact revealed by Whipple and his associates is that when homologous plasma was injected daily into healthy dogs over a period of several weeks, hyperproteinemia followed by proteinuria gradually developed, suggesting that the immediate tissue-utilization of the injected plasma was too sluggish to facilitate its rapid removal from the blood stream. Nonetheless the animals continued in a state of health and nitrogen equilibrium.

In the processes of absorption much of the digested protein is first deposited in the liver as so-called "transition" or "labile" protein (Whipple). From here it passes to the circulating blood for further metabolic transformation. Accumulating evidence indicates that some of this protein enters into tissue-enzyme systems as well as into the fixed or structural material of the cellular cytoplasm. But it is especially the transitional protein which consti-

tutes the "reserve protein" of the body's protein pool. From studies of Schoenheimer, Borsook, du Vigneaud, and their associates it is now evident that all tissue protein is in a state of equilibrium in which transaminating and transmethylating mechanisms are constantly operative. No tissue protein, therefore, is static; and chemical interchanges and replacements presumably occur continuously, thereby enabling the tissues to function, to maintain homeostasis, and to counteract deteriorative tendencies of various sorts.

In the course of disease, however, whether this be due to an inadequate ingestion of protein, to a loss of bodily tissue, or to interference with the continuous formation of tissue protein, depletion of the protein stores may gradually occur, eventuating in a general tissue-protein deficit. Thus a loss of plasma protein following a severe burn, in the course of nephritis or cirrhosis of the liver, or because of massive infection, with discharge of purulent material, may lead to a diminution of protein stores in the liver and elsewhere. In starvation or in malignancy there may be a similar effect. In time, therefore, the tissue deficit, particularly in the liver, may represent a marked reduction in the content of protein material normally available for passage to the plasma and other tissues. The accompanying hypoproteinemia indicates in a general way the degree of depletion of the tissue-protein stores. Moreover, as the "reserve" proteins become diminished there is a concomitant reduction of metabolic activity, because of a loss of considerable amounts of protein comprising important enzyme systems. In other words, as the *quantity* of tissue protein becomes reduced, the *rate* of protein metabolism

correspondingly slows down. The combined effect is manifested in the signs and symptoms of protein-deficiency disease, as mentioned previously.

Correction of such a pathologic situation obviously requires restoration of the depleted tissues to their normal state. This can best be done by preventing the further loss of protein and by reëstablishing the normal processes of protein synthesis by diet, by the use of blood and plasma, and in other ways. Recognition of these facts is responsible in large measure for some of the important recent advances in surgery and medicine, particularly as they relate to the treatment of burns, shock, malignancy, and liver disease.

Evidence substantiating the above ideas is now available from several sources. For example, the technique of electrophoresis has demonstrated that as the protein stores become reduced there are accompanying changes in the various components of the circulating plasma proteins. Of these the hypoalbuminemia is of interest with respect to the development of nutritional edema. The loss of globulin, moreover, particularly of the gamma fraction, is important in its relationship to maintenance of the mechanisms of acquired immunity, since much of the circulating antibody is found in this component. Thus in nephrosis there may be an almost total loss of plasma gamma globulin, a fact which may help to explain the decreased resistance of such patients to intercurrent streptococcic and pneumococcic infections. There is also a marked diminution of several globulin fractions in the serums of rats made protein-deficient by a prolonged low-protein diet; and it is in such types of animals that we

have demonstrated both a diminished ability to fabricate specific antibodies and to resist induced bacterial infections. It should be emphasized, however, that depletion of the tissue-protein stores requires a considerable period of time and that even in starvation the body holds onto its protein stores with a remarkable tenacity. Nevertheless, in the course of severe and prolonged protein depletion there result both an inadequate supply of building materials for the synthesis of antibody protein and a lessened rate of antibody synthesis.

A similar depletion of stored proteins can be shown, also, in other tissues, such as in the lymphoid organs, the bone marrow and in the carcass generally, all pointing to a profound loss of potential sources of cells and cellular products normally operating in times of stress Thus Asirvadham in our laboratory has reported that, whereas well-nourished rats responded with a leukocytosis after consecutive stimulations from injection of turpentine, protein-depleted animals developed a leukocytosis only after the first injection. Examination of the depleted bone marrows in such animals evidenced the paucity of a reserve supply of marrow cells which might otherwise be able to respond to the leukogenic stimulus. The diminution in amounts and activities of important enzyme systems suggests, also, a probable cause for the decreased ability of some depleted tissues to counteract the actions of certain poisons. The atrophy of important endocrine organs, notably the hypophysis, the thyroid gland, the suprarenals and gonads may also account for some of the manifestations of protein deficiency, due to a diminution in amounts and ac-

tivities of hormones essential for the maintenance of an active metabolism.

In view of the several ways in which severe and prolonged protein deficiency may adversely affect many bodily processes it is obvious that the effects of diet upon recovery should be thoroughly understood. This is particularly true in relation to many of the hazards of surgery and of debilitating disease where there is evidence of a lessened capacity of depleted tissues to counteract the noxious effects of anesthesia, trauma, and infection. It is important to learn, therefore, what conditions may influence repletion of a depleted animal, in order better to elucidate some of the problems of convalescence and of preoperative and postoperative care.

It will be recalled that when protein-depleted rats are fed an adequate repletion ration there is a rapid recovery of lost weight (due largely to regeneration of muscle mass), of plasma protein, of hemoglobin and of certain enzyme systems, and of recovery of the normal capacity to synthesize antibody protein and to resist infection. It should be noted that all of these events are important in the processes of convalescence. In the presence of active disease, however, whether because of loss of appetite or of interference from conditioning factors, it is not always easy or even possible to accomplish adequate repletion. Nevertheless the correction of pathologic conditions requires a clear understanding of the metabolic activities of relatively undamaged mechanisms.

Because a protein-depleted rat is hungry the animal will usually eat a good repletion ration avidly. This happens even when a proper mixture of amino acids is substi-

tuted for protein in the ration. However, a reduction in the amount of an essential amino acid in a ration will cause a rat quickly to lose appetite and to refuse to eat the complete ration (figure 18). In consequence he will fail to gain weight adequately (figure 19). Moreover, even

FIGURE 18*

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* The two protein-depleted rats were fed rations whose dietary nitrogen was supplied by the mixture of 16 amino acids, but with one animal receiving 20 mg. of tryptophan per day and the other only 10 mg. Each rat received 15 grams of ration daily. If the ration was consumed completely the rectangle for each ten-day period is black. Note in the lower rectangles the loss of appetite in the presence of a slight deficiency of trytophan.

after the rat has eaten well for several days and has made a good weight gain, omission of an essential amino acid from the repletion ration will be followed in a day or two by a loss of interest in his daily food. In other words the dietary lack of an essential amino acid leads quickly to acute amino-acid deficiency disease, characterized by loss of appetite and weight. This effect is all the more astonishing in view of the fact that no other dietary deficiency will cause such a rapid appearance of deficiency symptoms. On the contrary, for most dietary essentials the
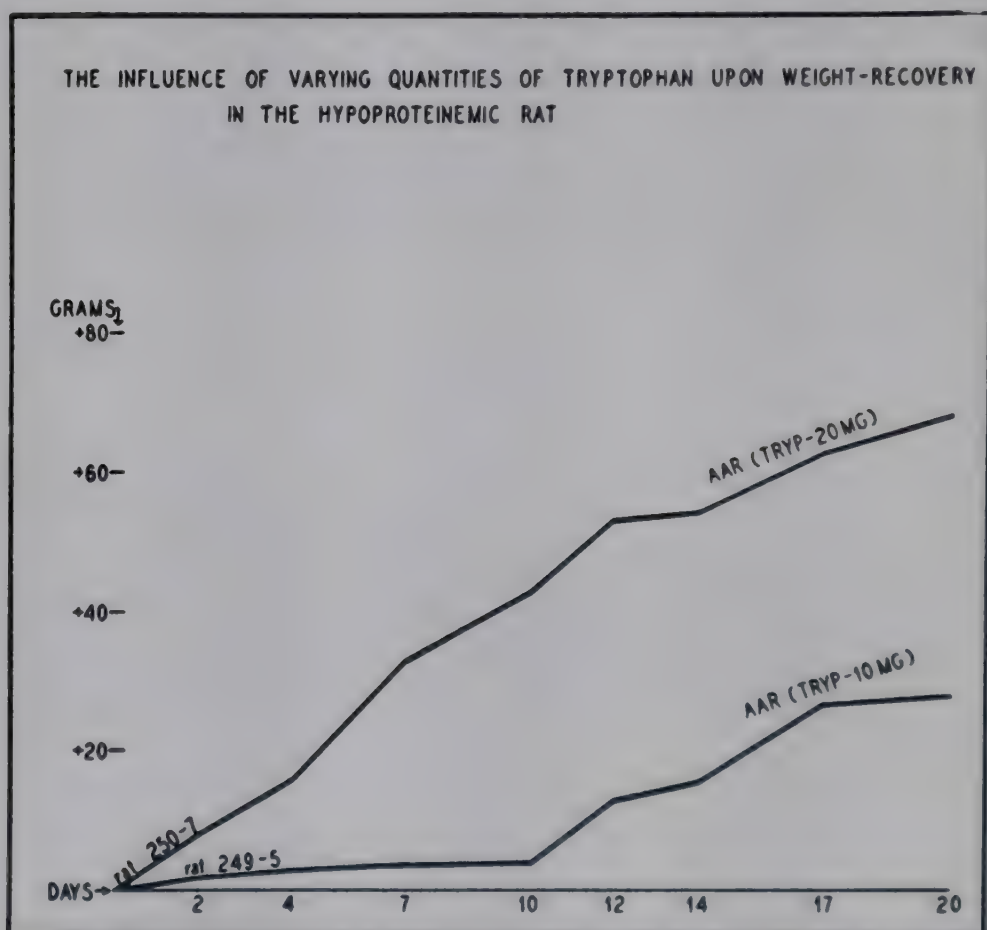

THE INFLUENCE OF VARYING QUANTITIES OF TRYPTOPHAN UPON WEIGHT-RECOVERY IN THE HYPOPROTEINEMIC RAT

FIGURE 19

Weight curves for the two rats whose food-consumption patterns are illustrated in figure 18.

tissue stores can be called upon in an emergency, but there is no adequate reserve supply of essential amino acids available, presumably, under similar circumstances.

The essential amino acids do not have to be ingested in order to demonstrate the effects of an amino-acid deficiency. For example, if protein-depleted rats are fed a repletion ration containing amino acids as the sole source of dietary nitrogen, good weight recovery will ensue even though one ration contains no lysine but the animal is injected subcutaneously twice daily with a solution containing an amount of lysine equivalent to that in the standard ration. However, when, after seven days, salt solution is substituted for the lysine solution, the animal quickly starts to lose weight (figure 20).
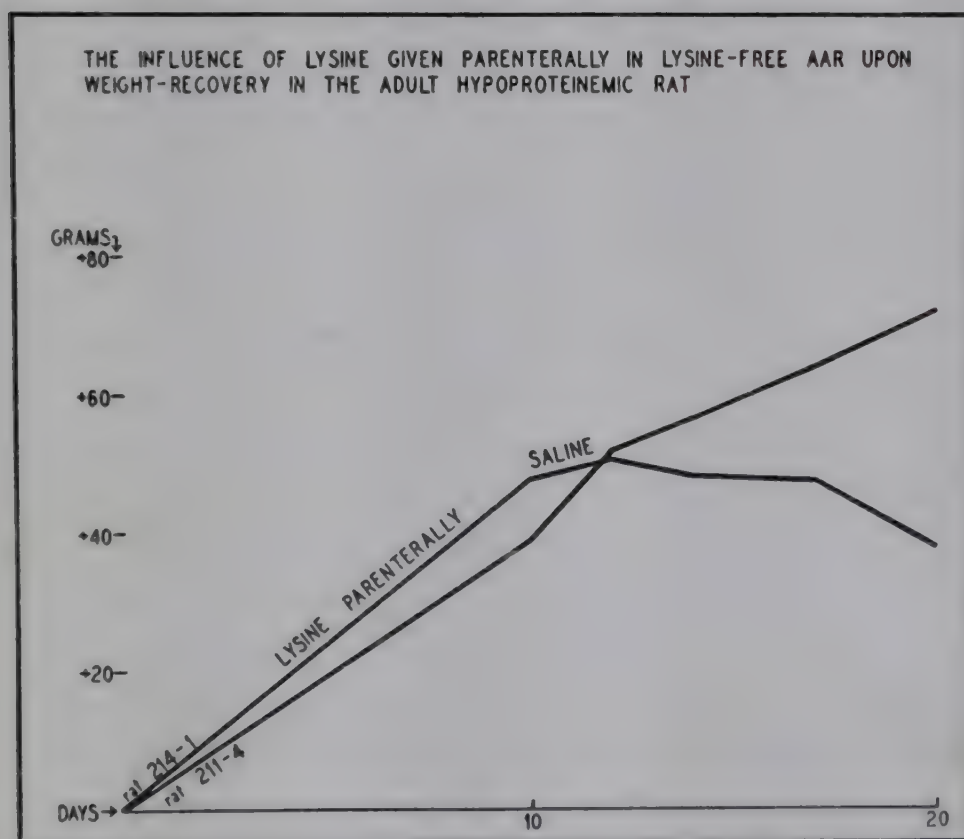

THE INFLUENCE OF LYSINE GIVEN PARENTERALLY IN LYSINE-FREE AAR UPON WEIGHT-RECOVERY IN THE ADULT HYPOPROTEINEMIC RAT

FIGURE 20

It might be thought that these manifestations of acute amino-acid deficiency are peculiar to the rat were it not for the fact that essentially similar effects occur in normal human beings, as has been demonstrated by W. C. Rose. Thus when he and his students fed rations in which eight essential amino acids constituted the main source of dietary nitrogen, they observed that the omission of any one of the eight led quickly to loss of appetite and of body nitrogen, and to the appearance of malaise. However, when the missing amino acid was restored to the diet the subject came back quickly into nitrogen equilibrium and to his previous state of health. These experiments demonstrate, therefore, that during the course of catabolism the subject becomes sick, with an associated loss of appetite. Evidently the dietary lack of one or more essential amino acids leads to a partial or complete blockage of protein synthesis and brings about the catabolic phase of metabolism, even in normal subjects. One might infer, therefore, that a sick patient losing tissue nitrogen should at least receive a diet adequate in all essential nutrients even though this may require the intravenous administration of a protein hydrolysate in order that all essential amino acids may be made simultaneously available to the depleted tissues.

These experiments, in rats and men, throw further light, also, upon the significance of the "negative nitrogen balance," in view of the fact that an inadequate intake of an essential amino acid can bring about such a rapid loss of body nitrogen. It may be, as has been suggested by others, that in the course of a negative nitrogen balance, as, for example, after a severe burn or trauma, or

as a consequence of infection, the metabolic utilization of a particular essential amino acid may bring about the "raiding" of tissues, with a casting-off of the other amino acids not similarly utilized. It has been suggested, for example, that in burns there may be a greater tissue utilization of methionine with the loss of other amino acids released but not used so avidly. But the question whether the negative nitrogen balance results from an augmented phase of catabolism or an impaired phase of anabolism, from tissue injury, has not yet been settled. At any rate, in healthy man the quantitative requirement for each essential amino acid is so precise that it is possible to establish the "daily requirement" for each of them; and W. C. Rose and his associates have done this for the eight essential amino acids required by man for maintenance. This important contribution will serve as a basis later for determinations of the altered requirements in abnormal states.

We have made similar determinations in adult white rats and have ascertained not only the individual requirement for each of nine amino acids essential for the maintenance of nitrogen equilibrium, but also for effective repletion of protein-depleted rats. By converting these figures to a common denominator, i.e., to the daily amino-acid requirements per kilogram of rat tissue, it has become evident that in the depleted animal there is a greatly increased utilization capacity for all the essential amino acids.

For the determination of the minimal amounts of essential amino acids required for maintenance of nitrogen balance and weight, normal rats were fed an amino-

acid ration in which one amino acid was progressively re-
duced in quantity until its smallest amount, in conjunc-
tion with the other fifteen amino acids in the ration, en-
abled the animal to remain in nitrogen equilibrium and
weight balance. In this way it was possible to ascertain for
each of nine amino acids the minimal amount necessary
daily per kilogram of body weight. For example, 23 milli-
grams of tryptophane were required, 32 milligrams of
histidine, 60 milligrams of lysine, and 170 milligrams of
isoleucine. In other words, a definite proportionality ratio
was evident, with tryptophane being utilized in the low-
est amount and isoleucine in the highest. Proof of the
validity of the findings was finally established by adding
to the basal ration a mixture of these nine essential amino
acids at these minimal levels. Rats fed this latter ration
also remained in nitrogen balance and maintained their
customary weights.

We have likewise determined the requirements for
effective tissue-protein repletion manifested by good
weight-recovery of protein-depleted rats. For example,
when such rats were fed our standard amino-acid ration
for ten days in equal amounts per day, the average weight
gain was approximately 46 grams, with a standard devia-
tion of around 5 grams. Therefore, the base-line require-
ment for weight recovery as 41 grams in ten days was es-
tablished; a rat accomplishing this gain was considered to
have performed satisfactorily. Knowing the daily food
consumption, one could then calculate the smallest
amount of any essential amino acid which, in conjunc-
tion with the other constituents of the diet, engendered
adequate weight recovery. When the amounts of each

amino acid so obtained were assembled, it was clear that the avidity of the depleted tissues for each essential amino acid was augmented from approximately onefold in the case of isoleucine to more than fourfold with respect to lysine (figure 21).

Of especial interest was the proportionality relationship of the different essential amino acids so determined, calculated in terms of tryptophane as unity. Thus, whereas for maintenance the highest utilization amounts were for isoleucine, valine, and leucine, with a relatively low amount for lysine, in active repletion the highest amounts were for leucine, isoleucine, lysine, and valine. This indicates that in the course of repletion the depleted animal displays an augmented capacity to utilize essential amino acids. This is not surprising in view of the overall loss of tissue-protein in the course of depletion and the presumed needs of depleted tissues for larger amounts of

MINIMAL DAILY REQUIREMENTS FOR ESSENTIAL AMINO ACIDS IN
A. NORMAL ADULT MALE ALBINO RATS
B. PROTEIN-DEPLETED ADULT MALE ALBINO RATS

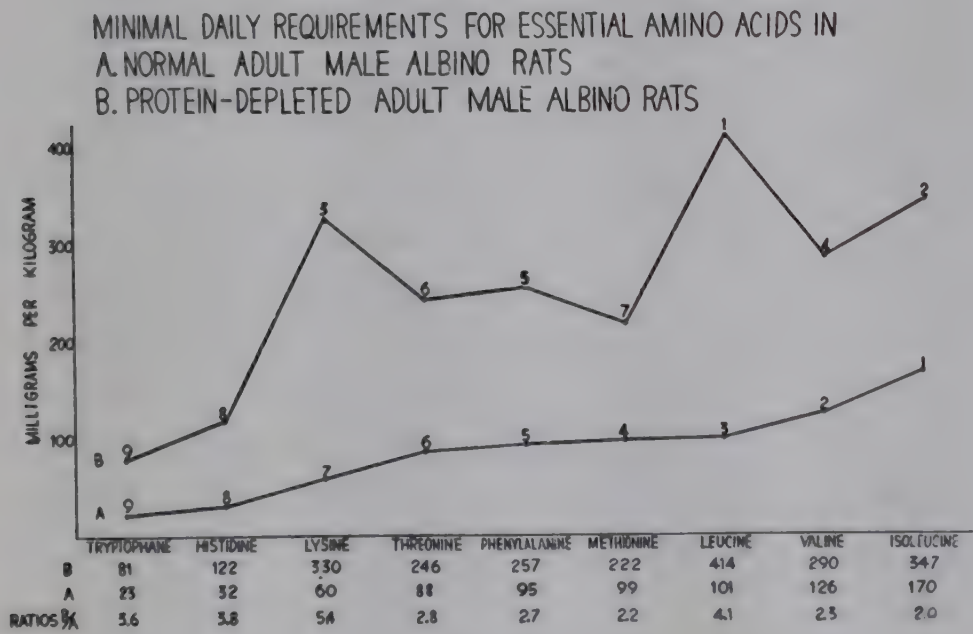|	TRYPTOPHANE	HISTIDINE	LYSINE	THREONINE	PHENYLALANINE	METHIONINE	LEUCINE	VALINE	ISOLEUCINE
B	81	122	330	246	257	222	414	290	347
A	23	32	60	88	95	99	101	126	170
RATIOS B/A	3.6	3.8	5.4	2.8	2.7	2.2	4.1	2.3	2.0

FIGURE 21

"building stones" for protein synthesis than are needed for maintenance alone.

In the reconstruction of depleted tissues it is probable that the needs of various organs differ, inasmuch as atrophy from undernutrition is most marked in striated muscle and least, for example, in nervous tissue. But since the total mass of striated muscle represents the largest source of tissue protein loss it is probable, too, that a large proportion of the absorbed dietary amino acids will go into the reconstruction of atrophic muscle. It would seem, therefore, that an understanding of the amino-acid composition of muscle should help to explain its reconstructive needs. Such information is now available. For example, Beach *et al.* have demonstrated a remarkable uniformity of amino-acid composition in striated muscles of various animals including beef, pork, lamb, fowl, and sea food. Moreover, they have shown that when some of the essential amino acids of meat are correlated on a molecular basis there is a definite proportionality relationship. Thus, using tryptophane as unity they have found a value for lysine of 10; for threonine, 7; for methionine, 4; and for histidine, 2. These findings suggest that, in the synthesis of muscle protein, for every molecule of tryptophane utilized there is a corresponding utilization of 10 molecules of lysine, 4 of methionine, etc. More recent analyses of beef muscle (Greenwood and Kraybill) demonstrate a similar relationship. In their comparative figures for nine essential amino acids, with tryptophane expressed as unity, the quantities of leucine and lysine were 8; of valine, 6; of isoleucine, 5; of threonine, phenylalanine, and methionine, 4; and of histidine, 3.

From these findings one might infer that in the regeneration of a large mass of atrophic muscle there is a greatly increased utilization capacity for all essential amino acids which enter into muscle structure; and from what has been said, these amino acids could be effectively utilized only if simultaneously available in adequate quantities and in the right proportions to one another. Thus, if depleted muscle has available only the amino acids released from the digestion of a poor protein such as gelatin or from a food in which the lysine has either been destroyed by processing or made unavailable for intestinal digestion, muscle reconstruction would be limited by the least amount of lysine available at the sites of synthesis. But since for every molecule of tryptophane approximately 10 molecules of lysine would presumably go into

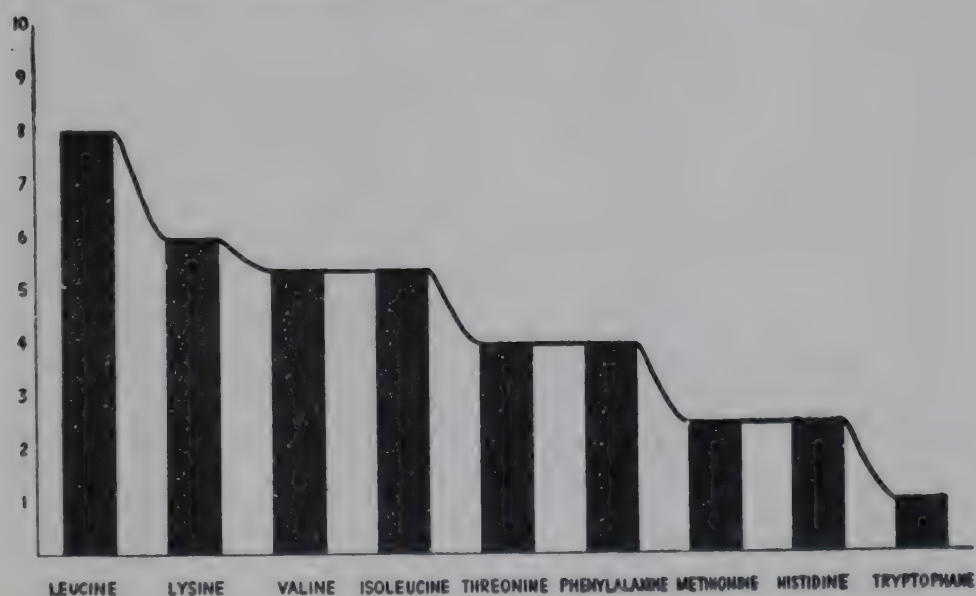PROPORTIONALITY PATTERNS OF ESSENTIAL AMINO ACIDS OF CASEIN HYDROLYSATE.

FIGURE 22

muscle tissue, it is obvious that muscle regeneration would be negligible, regardless of the richness of the diet otherwise, whether in calories, vitamins, or salts.

It is of further interest that, in general, amino-acid analyses of high-quality proteins indicate a remarkably similar proportionality pattern of essential amino acids (figure 22). This suggests that all high-quality proteins should be effective in supplying adequate amounts and proportions of essential amino acids for muscle reconstruction. A point of further interest is that after we had determined the minimal quantities of each essential amino acid utilized daily in the course of tissue repletion and had compared their proportionality relationships, we found again, with tryptophane expressed as unity, that they fell into the same general relationship as do the essential amino acids of a high-quality protein. It is not likely that this is mere coincidence; rather it suggests that depleted tissues have a differential avidity for individual essential amino acids because in no other way can they accomplish reconstruction in accordance with the patterns of synthesis presumably established for each tissue or each high-quality protein.

What inferences may be drawn from these facts with respect to protein needs in the ill patient? Here we are obviously hampered by a lack of precise data, since those now available refer mainly to needs for maintenance of nitrogen balance in the healthy subject. Nevertheless, after many years of investigation, it can be assumed that, solely for maintenance, the daily protein needs range somewhere between 20 and 30 grams of good-quality protein. If the average figure of 25 grams of protein is taken

as the minimal daily requirement in an otherwise adequate diet, how much protein is indicated in a depleted patient? Here the capacity of different tissues to utilize increased intakes of protein depends, presumably, upon the degree of protein depletion, the extent of various organ injuries, and the role of conditioning factors in relation to its effective utilization. But in any case tissue repletion would undoubtedly require a large assortment of essential amino acids and in the proper proportions for effective utilization by the synthesizing mechanisms. In the depleted rat we have seen that in order to satisfy the needs for lysine and leucine in the reconstruction of muscle mass the utilization capacity is from fourfold to fivefold that for maintenance. Therefore, in order to satisfy the greatest need one might suppose that a severely depleted patient should have at least five times his minimal maintenance requirement for protein, or 125 grams of high-quality protein daily. No doubt in many instances the need would be considerably less, possibly nearer 100 grams per day. In any case these values are in a range of practical acceptability and argue against the advisability of attempting to force huge quantities of protein into a sick patient, particularly since in many instances such large amounts may be far beyond the patient's utilization capacity.

Because dietary protein is expensive, whether taken orally or by vein, there is no need to waste it needlessly. Presumably much of it enters into structural combinations and what is not so utilized is deaminated and converted into glucose or excreted as urea. But protein is an uneconomical source of calories. Therefore, even in the

repletion of depleted tissues it would seem that only util-
izable amounts should be given. Indeed there is some in-
dication that even under conditions of repletion the
optimal need for any particular essential amino acid coin-
cides with the minimal need as well. For example, in ex-
periments in which we determined the minimal daily re-
quirement for each essential amino acid in the protein-
depleted rat it was of interest that quantities of a particu-
lar essential amino acid in excess of the minimal amount
had no additive effect upon weight recovery. Thus it will
be noted in figure 23 that in the determination of the min-
imal requirement of isoleucine additional amounts added
to the ration above the level of approximately 350 milli-
grams per kilogram did not engender further weight gain.
The weight curve instead quickly flattened in this range,
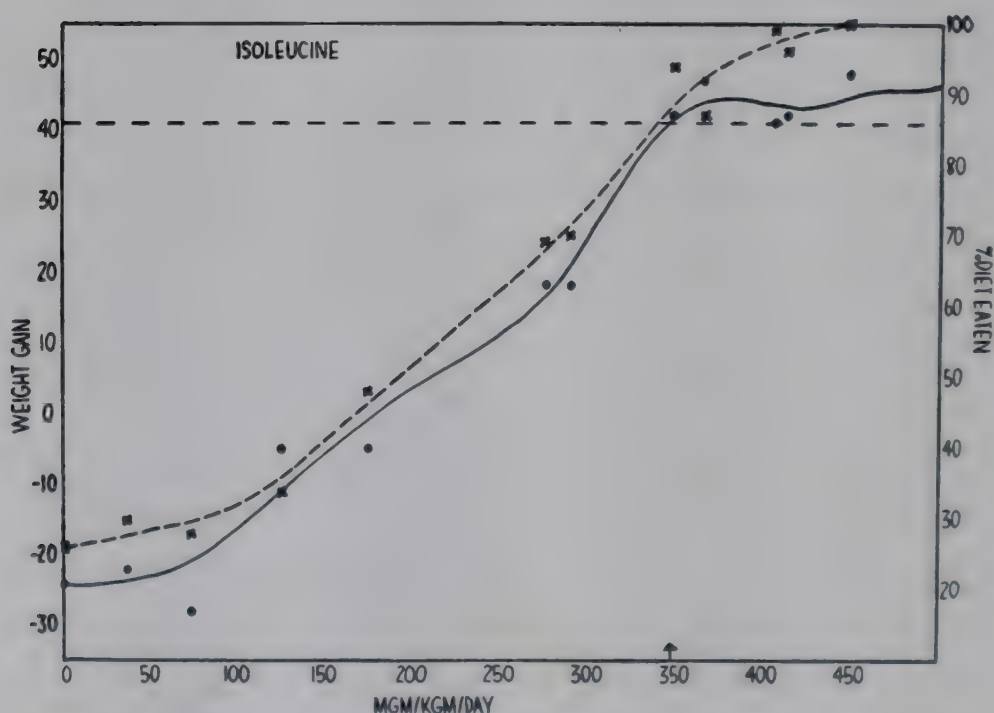suggesting that utilization of the additional isoleucine was

FIGURE 23

nullified by the limiting action of other components of the diet, presumably some limiting effect of the other essential amino acids. These findings would suggest, therefore, that above the point of optimal utilization it may be useless to attempt to secure effective protein synthesis, reëmphasizing the point that in the utilization of essential amino acids various limiting factors tend to regulate the overall mechanisms of synthesis.

The intravenous use of hydrolyzed protein has provided an additional means of counteracting the post-operative loss of tissue nitrogen, thus keeping the patient nearer nitrogen equilibrium until a proper diet can be given. The method, however, has presented additional problems; but now that the earlier difficulties from pyrogenic reactions have been largely eliminated the therapeutic problem is chiefly that of providing the most nutritive solution at the lowest cost. If the problem of caloric adequacy can be similarly solved it should be possible to keep post-operative patients in better strength and thereby hasten convalescence.

In the manufacture of these hydrolysates the makers have differed as to the proper method of hydrolysis. Enzymatic hydrolysis causes less injury to certain essential amino acids, especially tryptophane, which is frequently destroyed by acid hydrolysis. On the other hand, increasing evidence suggests that in the enzymatic hydrolysates there is a large residue of peptides and these, apparently, are used less effectively in the processes of protein synthesis. In consequence considerable amounts are lost in the urine. Now that efforts are being made to solve these problems better preparations may soon be available, thus

ensuring excellent tissue utilization of all essential amino acids.

The problem as to the best hydrolysate for intravenous use is still uncertain, and the proper methods of evaluation are not agreed upon. Customarily hydrolysates are evaluated in terms of their ability to establish nitrogen equilibrium or to engender plasma protein formation. Usually, however, too small amounts are given and under subcaloric conditions, to bring about much regeneration of plasma protein. This is due, presumably, to the fact that there is such an avidity of the depleted tissues for amino acids that the tissue needs must first be satisfied. Elsewhere we have made the suggestion that "hydrolysates should be evaluated in terms of overall synthesis of tissue protein. For example, the mere attainment of positive nitrogen balance in the ill or convalescent patient who is severely protein-depleted is not the goal of nutritional therapy. Rather, the objective should be to establish the highest possible level of physiologic nitrogen retention and thus ensure speedy repletion of depleted protein reservoirs. Similarly, the fabrication of limited quantities of plasma protein while the patient is losing weight and hemoglobin does not indicate adequate protein nutrition for the subject as a whole. Instead, the ideal protein hydrolysate would be one which most efficiently promotes maximal protein fabrication in all of the vital protein compartments of the body when fed or administered to the protein-deficient patient in practical quantities."

Because of the current interest in protein metabolism we are in a phase of excessive zeal with respect to the oral use of protein hydrolysates. Although there can be but

little question that a wider use of protein supplements will afford therapeutic advantages to ill patients, there is no good evidence that the proteins need be predigested. Indeed the experiences in some of the Nazi concentration camps indicated that almost moribund patients could metabolize protein concentrates quite effectively. There are few situations now known, therefore, in which oral hydrolysates are actually indicated, other than in conditions of allergy or where it is desired to have minimal amounts of residue in the digestive tract. Unfortunately, moreover, it has not been possible thus far to hydrolyze a protein and obtain a palatable product. The process of hydrolysis also increases costs to the patient. It would seem, therefore, that the burden of proof must continue to rest upon those who advocate the use of predigested protein. With reference to the cost, moreover, it is possible to buy skim milk powder in large quantities for protein supplementation at a fraction of a cent per gram of protein, whereas most of the hydrolysates may cost from ten to twenty times that much. Although the declining popularity of the oral hydrolysates warrants the making of these statements, it does not controvert in any respect the demonstrated value of supplemental protein concentrates in the treatment of abnormal states associated with a loss of tissue protein.

In summary, I believe it is apparent from these discussions that, despite the steady progress of the past few years, a great deal more needs to be learned about the intricate mechanisms of protein metabolism before we can speak too assuredly about the advantages and limitations of protein therapy. For example, but little is known

as yet about the influence of amino-acid imbalances upon protein utilization. Understanding of the role of "conditioning factors" is still limited, as is that, also, concerning the so-called "toxic destruction" of protein in pathologic states. The question of the differential absorption of amino acids in the course of intestinal digestion is still confused, and there is but little precise information about the mechanisms whereby excessive heat causes destruction of protein under various processing conditions. But in any case there can be no question about the fact that in the past decade there has been remarkably rapid progress in this important field of nutrition. It has been my aim in these Porter Lectures to epitomize in some degree a part of the record of this progress.

References

I

Cannon, P. R., Proceedings Amer. Fed. Soc. Exper. Biol. 7: No. 2, 1948.

Mendel, L. B., Nutrition: The Chemistry of Life, Yale University Press, New Haven, 1923.

Wissler, R. W., Woolridge, R. L., Steffee, C. H., and Cannon, P. R., J. Immunol., 52:267, 1946.

Brand, E., Kassell, B., and Saidel, L. J., J. Clin. Investigation, 23:437, 1944.

Madden, S. C., and Whipple, G. H., Physiol. Rev. 20:194, 1940.

Cannon, P. R., J. Immunol., 44:107, 1942.

Cannon, P. R., Advances in Protein Chemistry, Academic Press, Inc., 1945.

Wissler, R. W., Woolridge, R. L., and Steffee, C. H., Proc. Soc. Exp. Biol. and Med., 62:199, 1946.

Wissler, R. W., J. Infect. Dis., 80:250, 1947.

Wissler, R. W., J. Infect. Dis., 80:264, 1947.

Rose, W. C., Physiol. Rev., 18:109, 1938.

II

Cathcart, E. P., Biochem. J., 16:747, 1922.

Benditt, E. P., Humphreys, E. M., Wissler, R. W., Steffee, C. H., Frazier, L. E., and Cannon, P. R., J. Lab. and Clin. Med., 33:257, 1948.

Frazier, L. E., Wissler, R. W., Steffee, C. H., Woolridge, R. L., and Cannon, P. R., J. Nutrition, 33:65, 1947.

Wissler, R. W., Steffee, C. H., Frazier, L. E., Woolridge, R. L., and Benditt, E. P., J. Nutrition, 36:245, 1948.

Cannon, P. R., Steffee, C. H., Frazier, L. E., Rowley, D. A., and Stepto, R. C., Proc. Amer. Fed. Soc. Biol., 6, No. 1, 1947.

Melnick, D., Oser, B. L., and Weiss, S., Science, 103:326, 1946.

Rose, W. C., Science, 67:488, 1928.

Wissler, R. W., Benditt, E. P., Steffee, C. H., and Woolridge, R. L., Proc. Fed. Amer. Soc. Exper. Biol., 8:375, 1949.

REFERENCES

GEIGER, E. J., J. Nutrition, 34:97, 1947.

SHAFFER, P. A., and CLOEMAN, E., Arch. Int. Med., 4:538, 1909.

CANNON, P. R., Some Pathologic Consequences of Protein and Amino Acid Deficiencies. American Lecture Series, No. 27, Charles C. Thomas, Springfield, Illinois, 1948.

WHIPPLE, G. H., Hemoglobin, Plasma Protein and Cell Protein: Their Production and Interchange. American Lecture Series, No. 12, Charles C. Thomas, Springfield, Illinois, 1948.

III

WHIPPLE, G. H., Am. J. M. Soc., 196:609, 1938.

BENDITT, E. P., STEFFEE, C. H., HILL, T., and JOHNSON, T. L., Proc. Am. Fed. Soc. Exp. Biol., 8:350, 1949.

SCHOENHEIMER, R., The Dynamic State of Body Constituents, Harvard University Press, Cambridge, Massachusetts, 1942.

BORSOOK, H. and DUBNOFF, J. W., Ann. Rev. Biochem., 12:183, 1943.

DU VIGNEAUD, V., CHANDLER, J. P., COHN, M., and BROWN, C., J. Biol. Chem., 134:787, 1940.

ASIRVADHAM, M., J. Infect. Dis., 83:87, 1948.

ROSE, W. C., The Nutritive Role of the Amino Acids, The Science of Nutrition, The Nutrition Foundation, New York, 46, 1946.

ELMAN, R., Parenteral Alimentation in Surgery, Paul B. Hoeber, New York, 1947.

BEACH, E. F., MUNKS, B., and ROBINSON, A., J. Biol. Chem., 148:431, 1943.

STEFFEE, C. H., WISSLER, R. W., HUMPHREYS, E. M., BENDITT, E. P., WOOLRIDGE, R. L., and CANNON, P. R., Proc. Fed. Am. Exp. Biol., 8:369, 1949.

FRAZIER, L. E., WOOLRIDGE, R. L., STEFFEE, C. H., and BENDITT, E. P., Proc. Fed. Am. Soc. Exp. Biol., 8:355, 1949.

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